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Applicants: Shiguang Yu *et al.*	Examiner: Frank I. Choi
Serial No.: 09/683,003	Art Unit: 1616
Filing Date: November 7, 2001	Confirmation No.: 5003
For: **COMPOSITION AND METHOD**	Attorney Docket No.: 6601-00-HL

Declaration under 37 C.F.R. § 1.132 of Dr. Shiguang Yu

1. I am one of the inventors of the claimed invention in the captioned case serial number 09/683,003. I am also the first author of the Yu et al. reference being cited by the Examiner.

2. I hold a Bachelor of Medicine (BM, equivalent to MD in US) degree from Shanxi Medical University (China), a Master of Medicine (MM, major in nutrition and food hygiene) degree from Harbin Medical University (China), a Ph.D. in Nutrition from Wageningen Agricultural University (The Netherlands), and a MBA from Baker University (USA). I have worked for Hill's Pet Nutrition, Inc. (the assignee of the captioned case) since 1999, where I am currently a Principal Scientist. Before Hill's, I worked at another pet food company as a Senior Nutritionist for about two years, and was a Research Faculty member at the University of California at Davis in the field of feline nutrition for about a year after about two-year post-doctoral research. Prior to UC Davis, I was a research associate at the Faculty of Veterinary Medicine at Utrecht University, The Netherlands. A list of my scientific publications is attached as Exhibit A.

3. The claims as currently amended recite a method for increasing the rate of hair growth in a dog or cat comprising feeding the dog or cat a properly nutritious diet comprising from about 0.5 to about 4.5 mg of selenium per kg of the diet on the dry matter basis. I understand that previous versions of the claims before amendments stand rejected for lack of enablement.

4. One of the issues in the Examiner's lack of enablement rejection is the observation or allegation by the Examiner that the rate of hair growth reported in our study (for example, Table 1 at paragraph [0015] of our specification) was the same as or even less than that reported for the average rate of hair growth in beagles in our earlier publication.

5. We addressed the Examiner's observation in our amendment filed March 17, 2008. We argued that it is not scientifically appropriate to compare the growth rate previously reported with the growth rate in our specification, as the experimental conditions under which the previously reported rate was obtained are different. The Examiner dismissed our arguments as not supported by the evidence, arguing that "the authors in Yu et al. are the Applicants and as such would be in the position to know the comparative conditions between the working example and the conditions under which beagle hair growth was measured in the Yu et al. reference." Office Action of November 16, 2007 at page 12.

6. In response, I would like to clarify that the average hair growth rate data referred to in Yu et al. (Journal of Animal Physiology and Animal Nutrition 2006, 90:146-151) was gleaned from the text, Muller and Kirk's Small Animal Dermatology 6th Edition. *See*, pages 6, 65, of chapter 1 of this text provided herewith as Exhibit B. This text cites as a reference, Al-Bagdadi, F.A., et al. Am J Vet Res 38:611-616, 1977 (the reference is difficult to read in the attached Exhibit). Upon closer inspection, however, the cited Al-Bagdadi reference does not actually describe the hair growth rate of beagle dogs and I suspect that this reference was cited by the authors in error. I believe that the correct citation may be found in Gunaratnam, P. and Wilkinson, G.T., J. Small Anim. Pract. (1983) 24:445-453 which references Al-Bagdadi's Ph.D. thesis (Al-Bagdadi, F.A. (1975) *The Hair Cycle in Male Beagle Dogs*, Ph.D. Thesis. University of Illiniois, Champaign, Illinois (hereinafter referred to as the "Al-Bagdadi dissertation")). Applicants have recently obtained the Al Bagdadi dissertation. A copy of the Al-Bagdadi Thesis is provided as Exhibit C.

7. Although we might have observed a slower growth rate than described in the Al-Bagdadi dissertation, this does not lessen the validity of our data. Indeed, one of skill in the art might expect disparate results as different assay conditions, including season of the year,

location on the body from which the hair is sampled, and diet are known to influence hair growth rate. *See, e.g.*, Gunaratnam, P. and Wilkinson, G.T., J. Small Anim. Pract. (1983) 24, 445-453 (attached as Exhibit D).

8. In this case, as originally suspected, a review of the Al-Bagdadi dissertation indicates several major differences between our study (reported in our specification) and Al-Bagdadi's study. *See*, Al-Bagdadi dissertation, Materials and Methods section, pages 29-42. These differences could have significantly impacted the hair growth rate reported.

9. For example, Al-Bagdadi studied puppies and young adults (< 3 years) and only males while we used older dogs (> 7 years), both male and female. And most of these were either spayed or neutered. Although we are unsure of the impact that age and sex hormones may have on the rate of hair growth in beagle dogs, both age and sex hormones are known to have an effect on hair growth in humans.

10. Al-Bagdadi's dogs were kept outside during the day with the result that a seasonal change on hair growth rate was evident in his study; a daily hair growth rate in summer of 0.34 mm and 0.40 mm in winter was recorded. In contrast, our dogs were kept inside, under experimental conditions that included controlled temperature (21°C) and light cycle.

11. Al-Bagdadi's dissertation provides only basic information regarding what was fed to his dogs. Specifically, he does not report micronutrients (minerals and vitamins) in the food used in his study. I note that several of these nutrients, e.g., zinc and copper, are important for hair growth. Thus, unlike in our dogs, it is unclear from Al-Bagdadi's dissertation whether his dogs received a balanced amount of these nutrients.

12. Al-Bagdadi's sample size was relatively small - only 9 dogs in total (3 groups of 3) were studied. In contrast, we used 36 dogs in total (6 groups of 6). As a result, and given the degree of variation in hair growth measurement, Al-Bagdadi's data on hair growth rate may be less representative of the true average hair growth rate in a beagle.

13. In addition, the measuring methods employed in a hair growth rate study can contribute to differences in observed growth rates. One of skill in the art knows that measuring hair growth rate is particularly difficult and known measurement techniques can introduce error. For example, hair growth rate may be measured by shaving an area, reshaving the area as new hair grows back, collecting the shavings and directly measuring a random sample of shaved hairs using a microscope. *See, e.g.*, Comben, N., Observations on the Mode of Growth of the Hair of the Dog, Br Vet J 1951 May 107 (5) 231-235, copy of which is attached as Exhibit E; Gunaratnam, P. and Wilkinson, G.T., J. Small Anim. Pract. (1983) 24, 445-453, *see* Exhibit D. This technique is not entirely reliable, however, since not only is it subject to human error given the tedious nature of the process, it is also possible during shaving to recut a shaven hair, creating artificially short pieces.

14. In fact, Al-Bagdadi's dissertation indicates that the author tried the method described in Comben (1951) but rejected it given the "extreme variation" in hair segments after shaving. Al-Bagdadi attributed this variation as being the result of sampling a mosaic, or mix, of hairs growing at different rates, i.e., hairs in anagen, catagen and telogen. As a result, Al-Bagdadi chose to measure only the *longest* hairs (i.e., hairs in anagen) in the sample region in order to determine "the most rapid rate of growth". *See*, Al-Bagdadi dissertation at page 34. Specifically, using a ruler against the skin, Al-Bagdadi only measured 10 hair shafts and these 10 hair shafts were the fastest growing ones.

15. In contrast, given the imprecise nature of the commonly used methods to measure hair growth rate, we used a more sophisticated technique, taking pictures of new hair growth (using slides to press the hair to the skin in order to minimize errors caused by the angle formed between standing hair and skin), and scanning the negatives with a computer and employing image analyzing software to measure the length of hair in the image. *See*, Specification at paragraph [0013]. Unlike Al-Bagdadi, we measured all hairs in the camera view area, which typically included more than 50 hairs, thus our data represent an average of hair growth rate of all hairs counted, including slower growing hairs. Thus, our hair growth rate data actually may be *more accurate* than the rate previously reported by Al-Bagdadi.

16. I hereby declare under penalty of perjury that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: July 1st, 2008

Shiguang Yu, Ph.D., BM, MM, MBA

Declaration of Dr. Shiguang Yu

EXHIBIT A

Publications of Dr. Shiguang Yu

ARTICLES

1. Yu, S. (2008) Book chapter: Nutrients (vitamin section), Small Animal Clinical Nutrition (in press).
2. Yu, S. & Gross K.L. (2007) Dietary management of the three most common lower urinary tract diseases in cats. Hill's Symposium on Lower Urinary Tract Disease. Pp 53-57, April 18-20, Sunny Isles Beach, FL, USA
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ABSTRACTS

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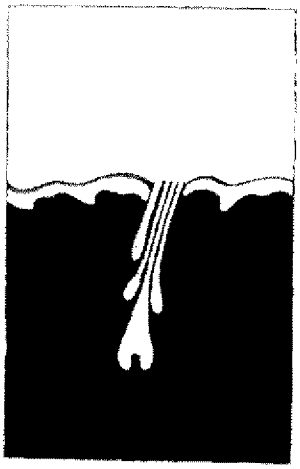
EXHIBIT B

Muller and Kirk's Small Animal Dermatology 6th Edition.
See, pages 6, 65, of chapter 1

Muller & Kirk's

SMALL ANIMAL DERMATOLOGY

6th Edition



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macrophages, fibroblasts, mast cells) in the immediate environment. In cats, there was no effect of repeated clipping on hair growth.[75]

Hair replacement in dogs and cats is mosaic in pattern because neighboring hair follicles are in different stages of the hair cycle at any one time. Replacement is unaffected by castration; it responds predominantly to photoperiod and, to a lesser extent, to ambient temperature. Dogs and cats in temperate latitudes such as the northern United States and Canada may shed noticeably in the spring and fall. Hair follicle activity, and thus hair growth rate, are maximal in summer and minimal in winter. For example, up to 50% of hair follicles may be in telogen in the summer, but this proportion may increase to 90% in the winter. Maximum hair follicle inactivity is reached earlier in female cats than in male cats.[75] Catagen hairs always constitute a small proportion of the total number of hairs, usually accounting for 4% to 7% of the total.[3, 180] Many dogs and cats exposed to several hours of artificial light (e.g., animals housed indoors) shed, sometimes profusely, throughout the year.[172, 180] Sinus hairs are *not* subject to a seasonal shedding, and are shed continuously as single hairs.[171]

Hair grows until it attains its preordained length, which varies according to body region and is genetically determined; it then enters the resting phase, which may last for a considerable amount of time. Each region of the body has its own ultimate length of hair beyond which no further growth occurs. This phenomenon is responsible for the distinctive coat lengths of various breeds and is genetically determined. In mongrel dogs, it was shown that hair growth rates varied at different sites and that the speed of growth was related to the ultimate length of the hair in each particular site.[66] For example, in the shoulder region, where ultimate hair length was about 30 mm, the average rate of hair growth was 6.7 mm/wk, whereas in the forehead region, which had ultimate hair length of about 16 mm, the growth rate was 2.8 mm/wk. Other investigators have reported daily hair growth rates in dogs of 0.04 to 0.18 mm (Greyhound)[23, 39] and 0.34 to 0.40 mm (beagle).[3] In the cat, daily hair growth rate has been reported to be 0.25 to 0.30 mm[17] or 62 to 289 $\mu g/cm^2$.[75]

Because hair is predominantly protein, nutrition has a profound effect on its quantity and quality (see Chap. 17). Poor nutrition may produce a dull, dry, brittle, or thin haircoat with or without pigmentary disturbances.

Under conditions of ill health or generalized disease, anagen may be considerably shortened; accordingly, a large percentage of body hairs may be in telogen at one time. Because telogen hairs tend to be more easily lost, the animal may shed excessively. Disease states may also lead to faulty formation of hair cuticle, which results in a dull, lusterless hair coat. Severe illness or systemic stress may cause many hair follicles to enter synchronously and precipitously into telogen. Shedding of these hairs (telogen defluxion; see Chap. 11) thus occurs simultaneously, often resulting in visible thinning of the coat or actual alopecia.

The hair cycle and haircoat are also affected by hormonal changes.[28, 65, 70, 115] In general, anagen is initiated and advanced and hair growth rate is accelerated by thyroid hormones and growth hormone. Conversely, excessive amounts of glucocorticoids or estrogens inhibit anagen and suppress hair growth rate. Dermal papilla cells, which are a mesenchymal component of the hair bulb, are considered to play a fundamental role in the induction of epithelial differentiation. These cells are morphologically and functionally differentiated from dermal fibroblasts and are thought to be the primary target cells that respond to hormones and mediate growth-stimulating signals to the follicular epithelial cells.[4, 84]

Obviously, the details of the regulation of hair follicle cycling and growth are extraordinarily complex and still poorly understood. The factors that control the hair follicle cycle are, in general, different from the factors that control hair follicle structure. Alterations in factors (e.g., hormones) controlling the hair follicle cycle result in *follicular atrophy*. Alterations in factors (e.g., morphogens) that control hair follicle structure result in *follicular dysplasia*.

Hair growth is a confusing subject that needs much research. It should be remembered that the haircoat of pet animals is a cosmetic or ornamental feature. Every effort

FIGURE 1–47. Arrector pili in normal skin from 9-year-old dog. Note numerous, variable sized, randomly scattered clear vacuoles within muscle section.

dermal atrophy (relatively acellular and avascular) and altered dermal collagen, elastin, and glycosaminoglycans; (4) atrophy of the subcutis; (5) attenuation of the eccrine and apocrine glands and decreased sebaceous gland secretion; (6) reduction of hair follicle density; (7) thinning, ridging, and lusterless nail plates; (8) decreased growth rate of the epidermis, hair, and nails; (9) delayed wound healing; (10) reduced dermal clearance of fluids and foreign materials; (11) compromised vascular responsiveness; (12) diminished eccrine and apocrine secretions; (13) reduced sensory perception; (14) reduced vitamin D production; (15) and impairment of the cutaneous immune and inflammatory responses.[65] Clinical correlates in humans of these intrinsic aging changes of the skin include alopecia, pallor, xerosis (dry skin), increased incidence of benign and malignant neoplasms, increased susceptibility to blister formation, predisposition to injury of the dermis and underlying tissues, increased risk of wound infections, and thermoregulatory disturbances.[65] Wounds in aged beagles and boxers healed less rapidly than in young dogs.[146]

Heterozygosity

Loss of heterozygosity is a genetic mechanism by which a heterozygous somatic cell becomes either homozygous or hemizygous because the corresponding wild-type allele is lost.[76] Loss of heterozygosity has been documented or postulated to be causative in various neoplasms (basal cell carcinoma, melanoma, squamous cell carcinoma), benign nevi (hamartomas), and a variety of dermatoses characterized by pronounced segmental occurrences.

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EXHIBIT C

Al-Bagdadi's Ph.D. thesis (Al-Bagdadi, F.A. (1975)
The Hair Cycle in Male Beagle Dogs

THE HAIR CYCLE IN MALE BEAGLE DOGS: A QUANTITATIVE, LIGHT AND ELECTRON MICROSCOPICAL STUDY

BY

FAKHRI ABDUL KARIM AL-BAGDADI

B.V.M., University of Baghdad, 1961
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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Veterinary Medical Science
in the Graduate College of the
University of Illinois at Urbana-Champaign, 1975

Urbana, Illinois

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

THE GRADUATE COLLEGE

MAY 1975

WE HEREBY RECOMMEND THAT THE THESIS BY

FAKHRI ABDUL KARIM AL-BAGDADI

ENTITLED THE HAIR CYCLE IN MALE BEAGLE DOGS: A QUANTITATIVE, LIGHT AND ELECTRON MICROSCOPICAL STUDY

BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

James E. Lovell
Director of Thesis Research

L. Meyer Jones
Head of Department

Committee on Final Examination†

James E. Lovell
Chairman

†Required for doctor's degree but not for master's.

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LIST OF ABBREVIATIONS USED IN FIGURES

APM	Arrector Pili Muscle
Bb	Bulb
BH	Black Hair
BM	Basement Membrane
BC	Basal Cells of the Matrix
BV	Blood Vessels
CC or cc	Cuticle of the Cortex
CF	Collagen Fibers
ch	Chromatin
CH	Club Hair
CI	Cuticle of Inner Root Sheath
CN	Common Neck
Co	Cortex
CoF	Cortex Fibers
CP	Cytoplasmic Process
CT	Connective Tissues
D	Desmosomes
DP	Dermal Papilla
Fb	Fibroblast
FCT	Fibrous Connective Tissue
FF	Fibrous Filaments
Fi	Fibrous Material
GG	Glycogen Granules
GM	Glassy Membrane
Go	Golgi's Apparatus
He	Henle's Layer
HG	Hair Germ Cell
Hu	Huxley's Layer
IG	Irregular Granules
Ke	Keratin
KF	Keratinized Fibers
MD	Melanocytic Dendrite
Me	Melanin Granules
MH	Main Hair
Mi	Mitochondria
Mu	Medulla
MV	Membrane Bound Vesicles
Mx	Matrix
Nc	Nucleolus
Nu	Nucleus
ORS	Outer Root Sheath
PM	Plasma Membrane
Ps	Polyribosomes
R	Ribosomes
Rer	Rough Endoplasmic Reticulum
SER	Smooth Endoplasmic Reticulum
SF	Subcutaneous Fat
SH	Secondary Hair
Tr	Trichohyalin Granules
UC	Undifferentiated Cells
WH	White Hair

I. INTRODUCTION

A basic understanding of the hair cycle is necessary as a background for the study of alopecia and dry hair coat in dogs. While a great deal of attention has been given to the hair follicle of laboratory animals, fur bearing animals and sheep, the hair follicle of the dog has not been the subject of detailed and controlled study. Information that is available in the literature concerning the hair follicle of the dog is general and gives little regard to the age, sex or breed of the dogs observed. There is also a great deal of information derived from observations of dog breeders based upon impressions and generalizations about shedding, hair growth, and seasonal variations in different breeds. It is difficult to separate fact from conjecture. There is very little reliable information available concerning the cyclic morphology of the canine hair follicle.

This work is an attempt to study the hair cycle of standardized defined dogs in which changes caused by environmental influences and endocrine control were known. Methods used included light microscopy, histochemistry, electron microscopy and macroscopic examination of the hair coat of living dogs.

It is hoped that this investigation will contribute to the available knowledge concerning the hair follicle and its cyclic changes and to the solution of some problems in canine dermatology.

II. REVIEW OF LITERATURE

A. General

The available literature written on the hair follicle of the dog is not plentiful. Most of the work deals mainly with the skin of the dog while the hair follicle is mentioned as a secondary part of the work. Webb and Calhoun (1954) in their study on the skin of mongrel dogs pointed out that the compound hair follicles are arranged in groups of three in the upper part of the dermis and in the deeper part of the dermis the component follicles of the three compound follicles spread into one large group of follicles. The development of the hair follicle has been investigated by Lovell (1955), in the Coonhound and Collie. He was mainly concerned with hair follicle development after birth and the histochemical and light microscopic changes that take place in the hair follicle gerontologically. Also Lovell and Getty (1957), reported on the arrangement of the canine hair follicle and pointed out that the number of secondary follicles increases between birth and 28 weeks of age. Creed (1953) in his work on the histology of mammalian skin referred to the hair follicles of mongrel dogs describing the histology briefly and pointing out that the cortex of the dog hair is relatively thick. In a comparative study of the skin of the dog and man, Schwartzman and Orkin (1962) classified canine hairs into tactile, guard and fine hairs and compared the hair of the dog with the human hair. Lovell and Getty (1968) in their review of the integument of the dog wrote very carefully on the hair coat of the dog in relation to hair development, compound hair and the morphological classification of the hair into six types, based on length, color and diameter. Lovell and Getty (1968) were the first to mention in their review that the hair cycle in the dog as it has been reported in other animals consists of three stages: anagen or active,

catagen or transitional and telogen or resting. Also Blackburn (1965) carefully reviewed the literature on the hair of cattle, horse, dog and cat. He cited Cair (1928) in classifying the hair coat of the dog into three classes: normal length type of hair as in the German Shepherd, short type of hair as in the Dachschund and Boxer and long type of hair as in the Poodle. Blackburn (1965), based on his own experience, stated that hair shedding in the dog occurs in the spring and in autumn.

Conroy (1969) in his light and electron microscopical study on the melanoblasts and melanocytes in canine fetal skin, observed the development of the main and secondary hair follicles and reported their appearance histologically. The literature on the skin and hair of the dog has been reviewed comprehensively by Muller and Kirk (1969), in their textbook on small animal dermatology. They included in their literature review the hair types, hair color and a description of the hair cycle stages as observed by the light microscope. In regard to hair follicle development, Conroy and Beamer (1970) reported the beginning of hair follicle development as early as in 28 mm. fetuses of Black Labrador Retrievers. Warner and McFarland (1970) in their report on the skin of the Beagle dog, described the group pattern of the hair follicles and the hair types classified according to size and shape into six kinds of hair.

B. Hair Follicle Development

The major anatomical details of the development of simple hair follicles have been well established in the human being (Pinkus, 1958). Little attention has been paid to the development of compound hair follicles, especially in the dog. Conroy (1968) in his study on Black Labrador Retriever fetuses found that the earliest primordial follicles of body hairs appeared at thirty days of gestation. He observed simple hair follicles on the back

in material collected from 40 day old fetuses. Conroy (1968) reported the development, in late gestation, of secondary hair follicles from the main follicles which had developed much earlier. Lovell (1955) in his investigation on the skin of Coonhounds and Collies studied the development of the hair follicles after birth. Lovell (1955) and Lovell and Getty (1957), explained that the hair follicles in the breed of dogs studied appear as simple hair follicles at birth and become compound hair follicles by 28 weeks of age. Lovell (1955) pointed out that the number of hairs per follicle increases with an increase in age. Lovell and Getty (1957) suggested that additional secondary hair follicles develop as buds from the original single follicle. This concept is supported by Blackburn (1965). A more recent study on the development of the compound hair follicles in dogs after birth was reported by Baker (1966) on mongrel dogs. He observed secondary hair follilces associated with primary follicles at birth. Baker (1966) provided evidence indicating that secondary hair follicles arise as projections from the primary follicles above the sebaceous gland ducts. This arrangement was also reported in the opossum by Lyne (1970). Dolnick (1969) reported to the contrary that no evidence could be demonstrated of new secondary hair follicles arising from established follicles in the mink.

C. Compound Hair Follicles

The hair coat of the dog has been described by different authors. Claushen (1933) described the hair in canine skin as arranged in groups with each group consisting of one main hair and three to nine accessory hairs. Epling (1953) reported as many as thirteen hairs passing through a common hair follicle orifice. Lovell (1957) observed seven to ten hairs coming out of each orifice. This was confirmed by Blackburn (1965). According to

Creed (1958), each main hair in the skin of the dog is surrounded by six to twelve lanugo hairs. Schwartzman and Orkin (1962) stated that the number of hairs that come out of each orifice of the dog skin is seven to ten. According to Muller and Kirk (1969) there are seven to fifteen hairs in a group, one is a main hair and the rest are called lanugo or under hair. Warner and McFarland (1970) in their work on the integument of the Beagle dog emphasized that there are six types and sizes of hair and pointed out that the hair follicle bundles are associated in groups of three. The references previously reviewed deal mostly with the microscopic anatomy of the hair and hair follicle of the dog. No references have been found in the available literature on the hair cycle and cyclic stages of the hair follicle in the dog. Due to this lack of information about the cyclic changes in the hair follicle of the dog; information on the hair cycle of other animals will be reviewed like the mouse, rat, guinea pig, seal, chinchilla, monkey, sheep and human being. The three cyclic stages of the hair follicle were named and defined as anagen, catagen and telogen by Dry (1926). Anagen is the active stage of the hair cycle, telogen is the resting stage of the cycle, and catagen is the transition from anagen to telogen. The classical description of the hair follicle deals with the anagen stage. Consequently, a detailed presentation of the parts of the hair follicle as they appear in anagen will be reviewed followed by a brief description of changes that take place in catagen and telogen.

D. Morphology of the Hair Cycle

1. Anagen Stage

The general morphology of the anagen stage hair follicle has been carefully examined in the human by Montagna and Ellis (1958), Montagna (1962), and Rath (1967); in the chinchilla by Lyne (1965); and in the mouse by

Parakkal (1969). The various layers of the hair follicle during anagen stage will be reviewed systematically starting with the outer most structures.

a. Connective Tissue Sheath of the Hair Follicle

The thickness of the connective tissue sheath varies according to the size of the hair follicle. Dolnick (1969) wrote that the connective tissue sheath is absent from lanugo hairs of man and speculated that it is absent in other very fine hair follicles. Montagna and Van Scott (1958) explained that in man, the connective tissue sheath is composed of a thin inner layer and a thicker outer layer. Both layers are composed mainly of collagenous fibers. By means of an electron microscope, Parakkal (1969) observed that the connective tissue sheath in the mouse contains spindle shaped fibroblasts that are loosely attached to each other by means of desmosomes. In regard to the hair cycle stages, Montagna and Van Scott (1958) pointed out that the connective tissue sheath changes in thickness and continuity during follicular cyclic activity.

Montagna and Van Scott (1958) wrote that the blood vessels of the hair follicle are embedded in the connective tissue sheath. They described plexuses of capillaries in the inner layer and a straight arrangement of arterioles parallel to the hair follicle in the outer layer.

b. Glassy or Vitreous Membrane

The structure of the glassy membrane is a hyaline, noncellular, two layered structure (Cooper, 1930 and Montagna, 1962) and is located between the outer root sheath and the connective tissue sheath of the hair follicle. Rogers (1957) and Parakkal (1969) stated that the two layers of the glassy membrane are collagenous and oriented at right angles to each other. The thickness of the glassy membrane varies along the length of the hair follicle. Schaffer (1933) pointed out that glassy membrane is hardly

visible around the upper part of the hair follicle. Montagna (1962) wrote that at this part of the hair follicle, the glassy membrane is a single layer. Schaffer (1933) wrote that the glassy membrane is thick around the middle part of the hair follicle and thickest around the lower third of the hair follicle where it is made up of two layers (Montagna, 1962). At the matrix region, the glassy membrane is very thin and impossible to demonstrate in the papilla cavity (Montagna, 1962).

c. Outer Root Sheath

The thickness and cellular morphology of the outer root sheath vary at different levels along the hair follicle (Montagna, 1962 and Parakkal, 1969). The greatest thickness of the outer root sheath occurs at the middle third of the hair follicle (Montagna and Van Scott, 1958). According to Roth (1967) it is multi-layered at this part of the hair follicle. Parakkal (1969) pointed out that the cells of the outer root sheath at the middle third of the hair follicle are cuboidal and contain large accumulations of glycogen particles.

The mitotic activity of the outer root sheath has been examined by different investigators. Montagna and Van Scott (1958) observed mitotic figures in the outer root sheath of human hair follicles at the upper part of the hair follicle where it blends with the surface epidermis. According to Straile (1965) mitotic activity occurs at the lower third of the hair follicle. He stated that the mitotic figures are seen during early anagen in the rabbit, mouse and silver guinea pig. The rate of these mitotic figures in the outer root sheath has been postulated by Bullough and Laurence (1958) and Chase (1958) to undergo a decrease in rate in the mature hair follicle.

In regard to pigment granules in the outer root sheath, Lyne (1965) observed melanin granules below the sebaceous glands and above the bulb in the chinchilla. Straile (1965) also reported the presence of melanin granules in the outer root sheath of silver colored guinea pig hair follicles. Both Lyne (1965) and Straile (1965) observed functioning melanocytes in the outer root sheath of the chinchilla and the silver colored guinea pig hair follicles.

By means of the electron microscope it has been observed by Parakkal (1969) that the outer root sheath cells above the bulb of the hair follicles in the mouse contain a compact Golgi complex, several mitochondria and rough endoplasmic reticulum. He also observed fibrils composed of filaments in different parts of the cytoplasm of the outer root sheath cells. According to Gibbs, (1938) the cells of the outer root sheath that rest against Henle's layer are rich in tonofibrils. He also stated that in the upper third of the hair follicle the cytoplasm of these outer root sheath cells becomes hyalinized and undergoes partial keratinization. The outer root sheath cells in the mouse hair follicle have large numbers of membrane bound vacuoles in their cytoplasm in the active stage of the hair follicle (Parakkal, 1969).

d. Inner Root Sheath

The inner root sheath is made up of three layers. Starting from the outside of the hair follicle the layers are: Henle's layer, Huxley's layer and the cuticle of the inner root sheath. It extends from the level of the sebaceous glands to the bulb of the hair follicle (Dalnick, 1969). These three layers are each one cell in thickness in most mammals (Montagna, 1962 and Birbeck and Mercer, 1957c), but in the large follicles of man,

Huxley's layer is two cells thick (Montagna, 1962). According to Auber (1950) Huxley's layer is one cell thick in the sheep, and is of variable thickness in the rabbit (Durward and Rudall, 1956).

The three layers of the inner root sheath keratinize in the upper third of the hair follicle. It has been found (Auber, 1950 and Montagna, 1962) that the cells of the inner root sheath acquire trichohyalin granules immediately after they arise from the matrix region during their maturation. According to Birbeck and Mercer (1957c) trichohyalin granules are transformed first in the cells of Henle's layer, because of these cells are nearest to the outer root sheath cells. While the cells of Huxley's layer differentiate more slowly than those of Henle's layer. According to Auber (1950) the cuticle layer is the last to keratinize. Auber (1952) and Montagna (1962), observed that above the middle region of the hair follicle, Henle's layer, Huxley's layer and the cuticle cells of the inner root sheath, all become fused into a solid hyalin layer. During the process of keratinization, filaments build up in the cytoplasm of the keratinizing cells in addition to the appearance of trichohyalin granules. The filaments are made up of fibrils (Parakkal, 1969). The filaments develop during the early stages of differentiation of the inner root sheath cells, (Parakkal, 1969). The number of filaments increases in the cytoplasm of the cells of the inner root sheath, during migration of these cells upward in the hair follicle (Parakkal, 1969). Different observations have been made in regard to the appearance of the filaments and the trichohyalin granules. According to Charles (1959), filaments appear before trichohyalin granules are formed, while Roth (1967), observed that both appear at the same time. He postulated that the fibrils that make up the filaments and

the trichohyalin granules increase in diameter as the cells of the inner root sheath rise up in the hair follicle.

e. Bulb of the Hair Follicle

The bulb of the hair follicle is the dilated part of its root. This dilated part of the bulb surrounds the dermal papilla during anagen and early catagen. The bulb of the hair follicle varies in shape and size. Montagna (1971) observed that the bulb shape in most rodents, insectivores and prosimians is elongated and is flattened in seals, while in pigs the bulb of the hair follicle is shortened.

Montagna and Van Scott (1958), for descriptive purposes, divided the bulb of the human hair follicle into a lower region and an upper region. According to Auber (1952), the two regions can be divided by drawing a line passing across the widest part of the dermal papilla which is capped by the bulb. The lower region of the bulb is made up of undifferentiated cells and the upper region consists of differentiated cells (Auber, 1952). These cells migrate up from the lower undifferentiated region (Montagna and Van Scott, 1958). As these cells move up in the bulb of the hair follicle, they undergo differentiation and maturation into the different kinds of cells that make-up the layers of the growing hair shaft and the inner rooth sheath (Montagna and Van Scott, 1958). The cells of the bulb are called matrix cells. Their morphology varies in regard to their location in the bulb and the stage of activity. According to Matoltsy (1958) the matrix cells of the active hair follicle in man are rounded in shape and are small in size. Montagna and Van Scott (1958) described the matrix cells of the human hair follicle which line the dermal papilla as being tall columnar in type. While Parakkal (1969), described the morphology of the matrix cells in the mouse as having spherical nuclei. The nuclei occupy almost the entire cell.

During electron microscopic examination of human hair follicles, Parakkal (1969) noticed that the cytoplasm of the differentiated matrix cells is scant in amount and contains few mitochondria. The undifferentiated matrix cells contain higher numbers of mitochondria and many stacks of smooth endoplasmic reticulum (Birbeck and Mercer, 1957a).

The pigment in the bulb has been studied by Montagna (1957). He observed that the melanocytes are present in the upper part of the bulb. Large numbers of these melanocytes are seen in the bulb over the upper half of the dermal papilla in black guinea pig hairs (Snell, 1972). According to Montagna and Van Scott (1958), the dendrites of the melanocytes at the bulb region are insinuated between the presumptive cells of the cortex and medulla.

f. Cuticle of the Hair

During the process of hair cell differentiation, the cuticle cells are the last to differentiate (Parakkal, 1969). They develop from the cells that are located in the center of the matrix cells (Birbeck and Mercer, 1957b). These cuticle cells become recognizable in the upper part of the bulb (Montagna and Van Scott, 1958). Their thickness has been pointed out by Birbeck and Mercer, (1957b) and Montagna and Van Scott (1958), as consisting of only one cell layer. These cuticle cells are regarded as the thinnest cells of the hair by Roth (1967). They elongate during their course of movement upward in the hair follicle (Parakkal, 1969) and become over lapped in the middle third of the hair. At the upper third of the hair, their nuclei disappear and they become hyalinized (Montagna and Van Scott, 1958) and the mature cells of the cuticle adhere to the cortex. They become fully keratinized at the upper third of the hair follicle (Montagna and Van Scott, 1958).

The presence of melanin granules in the cuticle of human hair have been denied by Birbeck and Mercer (1957b) and Montagna (1962). While Snell (1972), did observe melanosomes in the cuticle cells of guinea pig hair.

g. Cortex of the Hair

Morphologically the cortex has been described by Copenhaver (1964), as the main bulk of the hair and it consists of several layers of fusiform cells. They are cornified and cemented together at the middle third of the hair (Montagna and Van Scott, 1958 and Copenhaver, 1964). While Dolnick (1969), observed that the cortical cells are spindle-shaped at the upper part of the lower third of the hair. They are longitudinally oriented parallel to the long axis of the hair. The shape of the cortical cells at the bulb and above it are ovoid with spherical nuclei (Odland, 1964). Air spaces between the cortical cells have been described by Hausman (1944). He called these air spaces fusi. According to Matoltsy (1958), these fusi are filled with fluid at the region above the bulb and as the hair grows the fusi dry out and the fluid is replaced by air.

The presence of melanin granules in the cortex has been described by Montagna and Van Scott (1958). According to these authors, the melanin granules are aligned longitudinally in the cortical cells.

Electron microscopic study of the cortex shows that cortical cells project between adjacent medullary cells (Parakkal, 1969). He further explained that some projections of the opposite cortical cells meet each other. In regard to keratinization of the cortical cells, Odland (1964) explained that the fully keratinized cortical cells have a cytoplasm made up of low density filaments (Odland, 1964). These filaments are enclosed by a dense interfilamentous substance. This substance is an amorphous

matrix (Parakkal, 1969). The morphology of fibrous keratin and its appearance in the cortical cells has been examined by different investigators. Mercer (1958) is of the opinion that fibrous keratin appears first as clumps of fine filaments in the cortical cells. These fine filaments are made up of loose parallel fibrils in the mid bulb of the hair follicle (Birbeck and Mercer, 1957a). The filaments are wispy and scattered in the cytoplasm of the cortical cells (Parakkal and Matoltsy, 1964). The arrangement of these filaments is parallel to the long axis of the hair and they tend to aggregate near the edges of the cortical cells (Roth, 1967). At higher levels along the length of the hair the cortical fibrils increase in diameter, (Birbeck and Mercer, 1957a and Roth, 1967). They continued to explain that the cell organelles of the cortical cells become displaced and finally, at the upper third of the hair (Rogers, 1959), the cortical cells become tightly packed with fibrils and matrix substance.

h. Medulla

The medulla is the middle part of the hair. It varies in shape and continuity in relation to the type of hair. Montagna and Van Scott (1958), described the medulla as continuous or fragmented in the coarse hairs while in fine hairs, it is absent or discontinuous. Dolnick (1969) observed that the medulla is continuous in the hair of the mouse. According to Copenhaver (1964), the medulla is absent from fine hairs of the human scalp and is absent (Pinkus, 1958) in the fetal lanugo hairs. The hair of the pig has no medulla (Montagna and Yum, 1964). While in the horse, the medulla is regarded as a characteristic feature (Marshall, 1902).

The cellular structure of the medulla has been described by Roth and Helwig (1964b). They stated that the cells are cylindrical in shape. According to Montagna and Van Scott (1958) and Dolnick (1969),

the medulla is a ladder like structure. They wrote that the medulla is made up of one or more rows of cells.

The keratinization process of the medulla has been examined by means of the electron microscope. Parakkal (1969) noticed that spherical granules develop in the medullary cells during their differentiation. These spherical granules have been described by Auber (1952) as trichohyalin granules. Parakkal and Matoltsy (1964) regard these granules as medullary granules and they believe that the electron microscopic character of the trichohyalin granules is different from that of the medullary granules. These medullary granules vary in size. According to Parakkal (1969), the medullary granules increase in size during their progress up to the middle third of the hair follicle filling a large portion of the medullary cells. Roth (1967) added that the increase in size of medullary granules is due to partial coalesence of smaller granules. According to Roth (1967), these medullary granules are not associated with fibrils or any other cellular organelles, while Mercer (1961) and Rogers (1964) are of the opinion that the medullary granules undergo fibrous transformation during the process of medullary hardening. Roth (1967) observed fibrils in the medulla of murine hair. He explained that these fibrils are made up of filaments without a visible matrix. Roth (1967) described these fibrils as randomly oriented in the cytoplasm of the medullary cells at the middle and lower third of the hair follicle.

Vesicles occur in the medullary cells. These vesicles have been described by Parakkal (1969), as membrane limited vesicles in the cytoplasm of the medullary cells at the lower and the middle third of the follicle. According to Roth (1967) vacuolization occurs in the cytoplasm and in the mitochondria of the medullary cells at the middle third of the hair follicle.

Parakkal (1969) wrote that these mitochondria become swollen and their cristae lose their regular orientation.

i. Dermal Papilla

The dermal papilla is continuous with the connective tissue that surrounds the base of the hair follicle. It is capped by the matrix cells of the bulb during anagen. The shape and size of the dermal papilla varies in different hair follicles. Schnickel (1961) noticed that the follicles with large hairs have large dermal papillae, whereas those with small hair have small dermal papillae. According to Montagna and Ellis (1959), the dermal papilla of a growing hair is voluminous and he added that the cells of the papilla are far apart. In relation to the changes in the size of the dermal papilla, Straile (1965), pointed out that the dermal papilla of the rabbit hair follicle gradually increase in size until the middle of the growth period of anagen. After that there is a gradual decrease in the size of the dermal papilla. According to Moffat (1968) the dermal papilla in albino mice and the hair follicle bulb stop growing at the same time after which the hair shaft continues to grow at a lower rate.

The shape of the dermal papilla varies with the shape of the bulb (Parakkal, 1966). According to Kranzle (1912), the dermal papilla of the pig hair follicle is onion-like in shape. Multiple dermal papillae in one hair follicle has been observed by Montagna and Van Scott (1958). They pointed out that some hair follicles have more than one medulla and this causes, according to their interpretation, the dermal papilla to split at its tip into two dermal papillae.

The cellular structure of the dermal papilla has been identified as mesenchymal in nature (Van Scott and Ekel, 1958). The arrangement of these mesenchymal cells are described by Roth and Helwig (1964a), as forming

a cylindrical column. They also stated that these mesenchymal cells have scant cytoplasm. According to Parakkal (1969), the dermal papilla is mainly composed of a group of fibroblasts. The intercellular spaces of the dermal papilla are occupied by a hyaline ground substance (Montagna and Ellis, 1959). They also stated that some pigment granules are seen scattered between the cells of human dermal papilla during anagen.

The vascular supply to the dermal papilla has been the interest of many investigators. According to Montagna (1962), the dermal papilla of small hair follicles in the human being are devoid of blood vessels. They observed that the dermal papillae of large hair follicles are vascularized. While the dermal papilla of the mouse hair follicle does not have blood vessels, (Roth and Helwig, 1964a).

Following a study of the dermal papilla by means of the electron microscope during anagen, Porter (1964), stated that the fibroblasts have well developed rough endoplasmic reticulum and a prominent Golgi apparatus near the nucleus (Parakkal, 1969). While Roth and Helwig (1964a) noticed that the cytoplasm of the dermal papilla cells have few mitochondria and small Golgi apparatus. According to Parakkal (1966), the rest of the dermal papilla is occupied by ground substance and a few fibrillar components.

The dermal papilla cells are separated from the matrix cells of the bulb by a basement membrane. Roth and Helwig (1964a) pointed out that this basement membrane in the mouse dermal papilla is made up of a single layer of dense material. While the basement membrane of the guinea pig dermal papilla is multilayered (Parakkal, 1966). This multilayered basement membrane consists of several dense layers. According to Parakkal (1966), each layer is made up of fibrillar material. This material is embedded in a homogenous matrix. Parakkal (1966) added that the spaces between the

dense layers of the basement membrane do not contain any recognizable structures (Parakkal, 1966). Some of the cytoplasmic processes of the dermal papilla cells have been seen interdigitating with the basement membrane by Roth and Helwig (1964a), in the mouse dermal papilla.

The possibility of mitotic activity of the dermal papilla has been examined by Montagna (1958) and Moffat (1968). They concluded that the dermal papilla cells do not undergo mitosis.

2. Catagen

Catagen is a transitional stage which follows the active stage of anagen. According to Dry (1926), the change from anagen to catagen is very rapid in the hair follicles of rodents, and is relatively slow in man.

The morphological changes that occur in catagen have been observed and recorded by different workers. Montagna and Van Scott (1958) noticed that at the beginning of catagen, the inner layer of the glassy membrane becomes very thick in the human hair follicle. This observation has been reported also in laboratory animals by Wolbach (1951) and Chase (1954). Later, Wolbach (1951) in his study on the hair follicle of black mice in catagen stage wrote that the glassy membrane at the lower part of the hair follicle unusually becomes thick. Ellis and Moretti (1959) noticed that the inner surface of the glassy membrane develops a series of ridges. These ridges parallel invaginations in the outer root sheath. Chase (1954) and Wolbach (1951) observed that the connective tissue sheath of the mouse hair follicle during catagen becomes greatly wrinkled around the lower third of the hair follicle. Ellis and Moretti (1959) added that this wrinkling is less evident in human hair follicles. According to Montagna and Van Scott (1958) the connective tissue sheath hangs loosely around the lower part of catagen stage hair follicle. Some investigators have suggested that the

changes in the connective tissue sheath and the glassy membrane serve as a mechanism for squeezing the catagen hair follicle upward (Wolbach, 1951 and Chase, 1964).

The changes that occur in the outer root sheath of the hair follicle have been observed by Chase (1954) and Wolbach (1951) and Ellis and Moretti (1959) as characteristic corrugations. According to Ellis and Moretti (1959) these corrugations are one or two and they believe that these corrugations ring the hair follicle either partially or completely. At later stages of catagen hair follicles, the outer root sheath in the region near the bulb perishes and the remaining epithelial cells form part of the hair germ. Ellis and Moretti (1959) and Snell (1972) believe that the outer root sheath near the bulb of catagen hair follicles disintegrates completely. They also stated that the cells of Huxley's layer in catagen become atrophied. The root of the hair in catagen stage develop a brush like attachment to the cell mass which surrounds its base (Bullough and Laurence, 1958). This hair root becomes clavate (Montagna and Van Scott, 1958) with numerous keratinized fibers radiating from it. They also explained that the club part of the hair root becomes surrounded by a capsule of partially keratinized cells. Around these cells is a thick epithelial sac of indifferent epidermal cells. Few pigment granules are seen in the epithelial sacs (Montagna and Van Scott, 1958).

The lower part of the hair follicles also undergo changes during catagen. Roth (1967) wrote that during catagen, the cells of the bulb cease mitotic activity and the bulb decreases in size. According to Montagna and Van Scott (1958), the matrix cells of the bulb degenerate and the dermal papilla separates from,but remains in contact with, the base of the epithelial sac which contains the cells of the hair germ. Montagna and

Van Scott (1958) concluded that the hair germ is formed largely from the cells of the outer root sheath and the matrix cells play a minor role in establishing it. Ellis and Moretti (1959) explained that after the dermal papilla becomes free from the bulb it shows a short stalk of cells which connects the dermal papilla with the club hair follicle.

The dermal papilla of the catagen stage hair follicle undergoes alterations in its shape (Johnson, Butcher and Bevelander, 1945). It becomes narrow and elongated in the mouse hair follicle (Dry, 1926). While Straile, Chase and Arsenault (1961), observed that the dermal papilla shortens until it becomes a ball-shaped structure in the hair follicle of the black mouse. Chase (1958) explained that the dermal papilla leaves it's cavity as a rounded group of condensed dermal papilla cells.

In regard to the blood supply during catagen stage, Durward and Rudall (1949), observed that the blood circulation to the hair follicle wall in catagen does not change in the rat. Ellis and Moretti (1959) found that at the end of catagen in the human hair follicle there are no capillaries in the dermal papilla. They claim that catagen is initiated by the collapse of the blood vessels within the dermal papilla.

3. Telogen

Telogen is the resting stage of the hair cycle. The depth of the hair follicle into the dermis at this stage has been observed by Roth (1965), in albino mice. Roth reported that the hair follicle in telogen stage extends into the dermis almost to the level of the subcutaneous fat. Montagna and Van Scott (1958) wrote that the hair follicles in telogen stage are less than half their former length. They also described the connective tissue at this stage as being thick and fragmented. According

to Montagna (1962), the hair follicle during telogen stage is surrounded by a thin hyaline membrane.

The dermal papilla at this stage becomes a ball-shaped group of cells and migrates upward and rests underneath the capsule of the hair germ (Parakkal, 1969). A different arrangement was described by Roth (1965), in which the dermal papilla at telogen stage is a compact spherical group of mesenchymal cells. He regarded these cells as remnants of the dermal papilla. During telogen, the bulb of the hair follicle is very small or absent according to Roth (1967). He also pointed out that the inner root sheath of the hair follicle is not present during telogen stage.

The base of the club hair is formed by interdigitation of fully hardened cortical cells with the surrounding cells (Roth, 1967). He described the hardened cortical cells as being highly irregular and dentate with a brush-like pattern.

This stage has been carefully investigated by Roth (1965) by means of the electron microscope. He pointed out that the dermal papilla at telogen stage has spindle shaped peripheral cells, while the central ones are polygonal. He noted that the peripheral cells have foot-processes and between them are extracellular collagen fibrils. Roth (1965) believes that the cells of the dermal papilla have more cytoplasm during telogen stage than in anagen. He also described fibrils composed of filaments traversing the cells of the dermal papilla. His description of the dermal papilla cells included the observation of desmosomes along the plasma membranes which loosely connect adjacent cells.

E. Hair Growth

Hair growth in domestic animals follows two patterns: 1. Mosaic type growth; 2. Synchronous type. The mosaic type of hair growth has been

reported by Chase (1954), in the guinea pig. He explained that in the mosaic type, each hair follicle proceeds through its growth cycle independently and with its own frequency. According to Kligman (1959), the scalp of adult human beings has a mosaic pattern of follicular activity. He added that at any given moment neighboring follicles in the human scalp are at different stages of their cycle. While the synchronous type of hair growth has hair follicles growing at the same stage of their cycle over the same region or over the body of the animal (Chase, 1965). According to Ebling (1965), the hair growth and replacement is not normally synchronous over the whole body of adult mammals. It is also note worthy that the word "Wave" has been used often in describing the hair replacement pattern and growth in domestic animals. According to Chase and Eaton (1959), the wave type of hair replacement is defined as an orderly progression in time and space of the follicle entering the growth phase, anagen, of their cycle. The wave type of hair growth occurs in the mouse, rat (Chase and Eaton, 1959 and Johnson, 1958a), hamster, chinchilla and rabbit. Waves of hair growth do not occur in the guinea pig, Merino sheep, cattle or man (Ebling, 1965; Chase and Eaton, 1959; and Dowling and Nay, 1960).

F. Seasonal Hair Coat

The hair coat in mammals changes during various seasons of the year. Blackburn (1965) reported hair shedding in spring and in autumn in the dog. He added that the spring shedding of hair lasts about five weeks in daily groomed dogs. According to Muller and Kirk (1969), certain wild dogs shed generally in the spring and again in the fall. Shedding in cattle was observed by Berman and Volcani (1961). They wrote that spring shedding commences some six to ten weeks after the winter solstice. According to Nayman (1965), and Nayman and Nay (1961), four months are required for the

change from full winter to full summer coat in cattle. They also observed that less time is needed for the change from summer to winter coat. The hair coat of the Exmoor pony has been examined by Speed (1960). He wrote that the shedding of the winter coat takes place in the spring very quickly, but a cold wet season may delay it. Some animals have three moults during the year. Ebling (1965) wrote that the mountain hare in Scotland has three annual moults, autumn, winter and the spring moult. According to Rothschild (1942, 1944), the goat has three moults, an autumn, brown to brown, a winter, brown to white, and a spring, white to brown. Ebling (1965) observed that most breeds of sheep do not show a definite moult. The morphology of the hair coat in cattle has been examined in the winter by Hayman (1965). He pointed out that in cattle, the winter coat has the longest hair and coarsest compared to the summer hair coat.

The cyclic stages of the hair coat in cattle vary during the year. Dowling and Nay (1960) pointed out that the proportion of follicles in cattle at anagen stage reaches a peak in spring and autumn. They continued explaining that about 20% of the hair follicles are in the growth phase in mid-winter and mid-summer. Some hair follicles in cattle are in growth phase at all seasons of the year (Dowling and Nay, 1960). The number of these active hair follicles reach one peak in September and a high peak in March.

G. Hair Growth Rate

The rate of hair growth varies and is influenced by many factors such as race, sex (Hamilton, 1958), season of the year (Ono, 1963), age (Savil and Warren, 1962), region (Rook, 1965; Munro, 1966), nutrition (Ryder, 1958) and hormones (Savil and Warren, 1962). The rate of hair growth in the dog has been measured by Comben (1951). He found that the rate of hair growth

in Greyhound dogs is in two stages. A slow growth stage, 0.04 mm./day and a more rapid growth stage, 0.18 mm./day.

In regard to the stability of the hair growth, Fraser (1951), stated that the rate of hair growth in cattle is constant. The maximum rate of hair growth in rats is achieved in the first few days of growth (Johnson, 1958). According to Fraser (1965), the length output of wool is reduced when there is increased fiber cross-sectional area. The regional effect of the body of the animal on the rate of hair growth has been examined by (Lyne, 1965). He reported the growth in length of hairs in the chinchilla is 0.7 mm./day on the lateral aspect and 0.5 mm./day on the dorsal aspect of the trunk. According to Myers and Hamilton (1951), the hair follicle of human scalp produces about 0.35 mm. of hair shaft per day. In regard to age and season, Rook (1965) found that the human scalp hair grew more rapidly between the ages of fifteen and thirty and at a slightly higher rate in summer than in winter. According to Ferriman (1971), the rate of hair growth of human hair is slightly greater in children than in adults. Trotter (1924) measured the daily growth of the hairs of the legs in human beings and noted that it increased with advancing age between seventeen and forty five years old. Myers and Hamilton (1951) like-wise reported a decrease in the growth rate of axillary hairs with age. Pelfini, Cerimele and Pisanu (1969) failed to find significant changes with age in the hair growth rate of humans. Their mean values ranged from 0.33 to 0.39 mm./day. Dolnick (1969) calculated that the rate of hair growth in Macaca Mulatta is 0.35 mm./day. Forbes (1969) believes that hair length is affected by age and breed in swine. He calculated that the rate of hair growth is about 3 mm./week.

H. Histochemistry

1. Alkaline Phosphatase

The dermal papilla of the canine hair follicle in anagen stage and its reaction to alkaline phosphatase has been examined by Lovell (1955). He noticed that all the dermal papillae of the active hair follicles in dogs which he studied from birth to 28 weeks of age contained alkaline phosphatase. While in the hair follicle of the rat and mouse, alkaline phosphatase is present to a slight degree in the early epithelial bud of the hair follicle (Butcher, 1951). In the human fetus, Serri, Montagna, and Huber (1963), have demonstrated the appearance of alkaline phosphatase concomitant with the earliest signs of hair follicle formation. They added that the amount of the enzyme increases in the dermal papilla when the follicle elaborates the hair. According to Hardy (1952), in the mouse, at the beginning of the growing phase the enzyme reaction in the dermal papilla and in the outer root sheath around the bulb is very strong. In regard to the human fetal life, Serri, Montagna and Huber (1963), indicated that alkaline phosphatase appears early in fetal life in the dermal papillae of anagen stage follicles and it also appears in the endothelial cells of the blood vessels. Moynahan, Sethi and Brookes (1972) in their investigation on the developing skin of rats noticed that the primordia of the hair follicle, at 15 days of gestation, were associated with slight alkaline phosphatase activity. They also found that the blunt ends of the hair follicles growing into the dermis showed alkaline phosphatase activity. Moynahan, Sethi and Brookes (1972) explained that seven days after birth in rats, alkaline phosphatase activity was found only in the blood vessels of the dermal papillae.

The location of alkaline phosphatase reaction has been mostly observed in the dermal papillae. In the dog, Lovell (1955) observed the highest concentration of alkaline phosphatase in the dermal papillae. Achten (1959) also agreed that the dermal papilla has an intense reaction for alkaline phosphatase in the rat. Kong (1968) reported that alkaline phosphatase activity is present diffusely in the dermal papilla of human hair follicles. He also pointed out that heavy deposits of alkaline phosphatase occur in the capillaries and in the bottom of the papilla cavity. According to Lovell (1955), the nuclei of the hair matrix cells of dogs hair follicles have less evidence of alkaline phosphatase than the dermal papilla and probably represent a diffusion artifact. The enzyme activity also appears abundantly in the connective tissue which surrounds the hair bulb in the rat (Kong, 1968). While Montagna (1962) observed that the dermal papilla and the endothelium of the blood vessels which surround the hair follicle contain alkaline phosphatase. According to Braun-Falco (1958), alkaline phosphatase is present in the middle portion of the outer root sheath of the active hair follicle.

In the vascularized papillae observed by Montagna (1962), the endothelium of capillaries contain alkaline phosphatase while the surrounding connective tissue is weakly reactive to alkaline phosphatase. During electron microscopic observation, Bell (1967) noticed that alkaline phosphatase is located on and near the plasma membranes of the dermal papilla cells. Montagna (1957) postulated that there is an inverse relationship between the amount of vascularity and the strength of the reaction for alkaline phosphatase in the dermal papilla. According to Montagna, (1958) the follicles of the bald scalp of human beings are much more reactive for alkaline phosphatase than large follicles inspite of the

fact that blood vessels are absent. Alkaline phosphatase in active hair follicles have been examined by Braun-Falco (1958). He noticed a strong reaction for the enzyme in the dermal papilla of active follicles of the human being. While the mature cells of the dermal papilla of the rat did not show a reaction to alkaline phosphatase (Moynahan, Sethi and Brookes, 1972). The work done by Kopf (1957) showed that in adult human skin, alkaline phosphatase activity is strongly positive in the dermal papilla of the active hair follicle. He also showed that this strong reaction of the enzyme vanished from the dermal papilla during the telogen stage. A similar statement was made by Hardy (1952); Johnson and Bevelander (1946), that the enzyme activity disappears from the dermal papilla of the hair follicle during the resting stage. The dermal papilla in the rat and mouse was examined by Butcher (1951). He pointed out that the amount of the enzyme is negligible in telogen stage in the rat and the mouse hair follicle. While Montagna (1962) stated that regardless of the state of activity of the hair follicle, the dermal papilla of the smallest hair follicle is always reactive for alkaline phosphatase. He also indicated that in progressively larger follicles, the dermal papilla becomes less reactive to the enzyme.

Different points of view have been expressed in regard to alkaline phosphatase and its function and fate. Braun-Falco (1958) speculated that the enzyme in the dermal papilla might be related to the transport of organic substances to the bulb during differentiation and growth. He also suggested that the presence of 5-nucleotidase activity might indicate the importance of this enzyme in the metabolism of nucleic acid. Johnson and Bevelander (1946), based on their studies on the alkaline phosphatase and glycogen in the hair follicle of pig embryos, assumed that the enzyme is

concerned with glycogenesis in these structures. Braun-Falco (1958) did not believe that the statement is justifiable, on the basis that alkaline phosphatase is found in greatest concentrations in the dermal papilla during the growth phase, at which time it is free of glycogen.

In regard to the function of alkaline phosphatase, Bell (1967) speculated that alkaline phosphatase acts as a catalyst for the transformation of nutritive materials for the maintenance of epithelial cells. Hamilton (1965) has demonstrated that alkaline phosphatases are essential for feather follicle development.

2. Glycogen

The presence of glycogen granules in hair follicles has been demonstrated by different investigators. The cells of the outer root sheath of the hair follicle at anagen stage contain large quantities of glycogen granules in the mouse (Hardy, 1952) in the Coonhound and Collie hair follicles (Lovell, 1955), and in human beings (Montagna, 1962). These glycogen granules are localized in the middle third of the hair follicle in large amounts (Lovell, 1955, and Montagna, 1962). The morphology of these glycogen granules is filamentous in shape (Montagna, 1962). There is some difference of opinion concerning the presence of glycogen in the hair follicle during telogen. Shipman, Chase, and Montagna (1955) observed that hair follicles at telogen stage in most mammals contain no glycogen. Montagna (1971) on the other hand found that glycogen is present in the cells of the epithelial sac around the club hair in telogen stage of man and most primates. The presence of glycogen granules in the outer follicle root sheath of the human being at telogen stage was confirmed by Montagna, Chase and Lobitz (1952). Also different opinions have been expressed on the amount of glycogen granules in catagen stage. Braun-Falco (1958)

reported large quantities of glycogen granules during anagen stage followed by disappearance of these granules in catagen stage. While Parakkal (1970), in his study on rat and mouse hair follicles, observed large accumulations of glycogen granules in the cells that surround the club hair during catagen.

Glycogen granules have also been seen in the matrix cells and the dermal papilla of the hair follicle. Lovell (1955) observed glycogen granules in the matrix and dermal papilla of active hair follicles of Coonhounds and Collie dogs during their first week of life. At later ages, glycogen granules were not present in the dermal papilla. According to Roth (1967), glycogen granules in the matrix cells are very sparce or absent. While Pinkus (1958), reported that the cells of the matrix are always free of glycogen granules at the bulb of the hair follicle. According to Parakkal (1969), the cells of the medulla of the mouse hair follicle at anagen stage, contain large accumulations of glycogen near the nucleus. In regard to the presence of glycogen granules in the dermal papilla, Parakkal (1966) observed occasional fibroblasts in the dermal papilla of the guinea pig hair follicle which contained PAS-positive material and he believed that it was glycogen.

It has been mentioned in the literature that a PAS-positive diastase resistant reaction occurs in the glassy membrane and the dermal papilla of the human hair follicle (Montagna, 1962), and in the basement membrane of the chinchilla hair follicle (Lyne, 1955). Also Parakkal (1966), observed that the dermal papilla of the guinea pig hair follicle is PAS-positive.

III. MATERIALS AND METHODS

Nine male Beagle dogs were used in this investigation. Females were not included in order to avoid the possibility of variations due to hormonal effects on hair growth during the reproductive cycle. The nine dogs were housed at the Old Small Animal Clinic on the campus of the University of Illinois and were kept in outside runs from 9:00 A.M. to 4:00 P.M. each week day during the entire year of sampling and observation. On weekends and holidays they were outside for only two to four hours each day.

The dogs were divided by age into three groups, (Table 1). At the beginning of the experiment, three dogs were two weeks of age, HE-1, KV-2 and LL-3, the second group 12 months old, GN-4, KF-5, (Fig. 1) and CR-6, and the third group 21 months old, EN-7, GH-8 and FL-9, (Fig. 2). All dogs were pure bred Beagles, purchased from Laboratory Research Enterprises, Inc., Kalamazoo, Michigan. In order to obtain dogs with sufficient white for the purposes of the study, two dogs (GR-8 and FL-9) were taken from a special strain of Beagles with a predisposition to epilepsy.

Sampling of each group was carried out for a year. By putting the groups together an evaluation of the dogs hair coat for up to 32 months of age by means of biopsies and 36 months by the combing method was achieved. During the weekly sampling, all the visual observations related to the hair coat and the dogs health were recorded. The dogs were fed a commercially prepared food once daily which contained, 25.76% protein, 8.70% fat, 4.1% fiber, 8.76% ash, and 49.70% nitrogen-free extract.* Following clipping of the hair (Fig. 3) skin biopsies were collected (Figs. 5 and 7) with the

*Information from Wayne Dog Food Corporation

aid of a prototype punch biopsy gun,[b] (Fig. 4) every month from the lateral side of the saddle region during the year of sampling. The biopsy wounds were closed with 20 mm. wound clips (Fig. 6). The biopsy site was alternated each month between the right and left side of the dogs.

The epileptic dogs did not go into seizure during collection of hair samples by combing during the one year of sampling. At the time the first biopsies were collected the dogs did not object to the procedure and no tranquilizer or anesthesia was used. During the second collection the dogs manifested anxiety and Innovar-Vet (Pitman-Moore)[c] was used as a tranquilizer. At the second time of biopsy collection, GN-8 and FL-9 went into epileptic seizures when the wound clips were applied. After this, Innovar-Vet was not given to tranquilize the epileptic dogs due to its action as an epileptic stimulator.

At the time of the third and subsequent biopsies, the epileptic dogs were given intramuscular injections of acepromazine maleate (Ayerst),[d] as a tranquilizer and the non-epileptic dogs were given intramuscular injections of Innovar-Vet. Acepromazine did not precipitate epileptic seizures. After biopsies were collected the specimens were divided into 3 pieces for formalin, alcohol and gluteraldehyde fixation (Figs. 8 and 9).

Following routine dehydration and clearing, the pieces of the biopsies fixed in 10% neutral formalin were embedded in Bioloid parafin embedding compound (Scientific Products)[e] in a Fisher-Tissuematon, Model TMF

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[b]Prototypes Inc., 10449 Metropolitan Ave., Kensington, Maryland 20795

[c]Manufactured by Pitman-Moore, Inc., Washington Crossing, New Jersey 08560

[d]Manufactured by Ayerst Laboratories, Inc., New York, New York 10017

[e]Scientific Products, 1210 Leon Place, Evanston, Illinois

autotechnican. Serial sections were cut from each block (at least 12 slides were prepared from each block with 4 to 14 sections on each slide). The sections were cut parallel to the slant of the hair follicles. Due to the compound type of canine hair follicle and the divergence of follicle angles it was difficult to section all the hair follicles in a perfect longitudinal plane. This was especially true when there was curling of the tissue during processing in dogs that had a thin fat layer in the subcutaneous tissue. Staining was done with Harris Hematoxylin and Eosin (Luna, 1968). The light microscope sections were photographed using Kodachrome II 35 mm. color slide film (Photolamp) and polaroid 3¼ x 4¼ black and white positive negative print film.

The portion of the biopsy that was used for histochemical study was fixed in 80% chilled alcohol for 48 hours and then passed through increasing percentages of alcohols and xylol and in two changes of low melting point paraffin for a total of three hours. The method used to recognize the presence of alkaline phosphatase activity was Gomori's calcium cobalt method (Gomori, 1952). The sections were cut at 10-40 microns in thickness. Following deparaffination, they were incubated for six hours at 37° C in a medium of the following composition:

10 ml----------3% Sodium glycerophosphate
10 ml----------2% Sodium diethyl barbiturate
20 ml----------2% Calcium chloride
1 ml----------5% Magnesium sulphate
5 ml----------Distilled water

Following incubation in this mixture, sections were rinsed in distilled water, treated with 2% cobalt nitrate for 3-5 minutes, rinsed in distilled water and treated with dilute solution of yellow ammonium sulphide for 1-2 minutes. This solution is prepared by adding three drops of ammonium

sulphide in 200 cc of distilled water. Finally the sections were dehydrated in two changes of 95% alcohol and two changes of absolute alcohol, cleared in xylene and were mounted in permount.

The presence of glycogen was examined in the alcohol fixed tissue by means of the McManus method for the Periodic Acid-Schiff Reaction (PAS) according to Luna, (1968). Sections were placed in 0.5% diastase for thirty minutes in an incubator at 37° C as controls for glycogen identification. The diastase dissolves the glycogen granules and removes them from control sections. Glycogen which was not exposed to diastase treatment appeared as a very dark red or purple granular material.

For light microscopic observations the main and secondary hair follicles were identified and described. The three hair cycle stages: anagen, catagen, telogen were counted in five slides for each month for all the dogs during the twelve months of sampling. Each slide contained 4 to 14 sections. The counts of each stage of the hair cycle were totaled for that month and the percentage of both main and secondary hair follicles combined for each stage was determined. The resulting percentage was regarded as the percentage of that stage in that month. The same procedure was followed for all of the twelve months. These percentage results were recorded for each dog and plotted on graphs. For low power studies of the hair follicles, in a few instances, 100 to 200 micron sections were prepared by cutting thin slices from the formalin fixed blocks with a razor blade. These sections were dehydrated, cleared in xylene and mounted on slides under cover slips with permount (Fisher Scientific Co.)[f] prior to examination.

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[f]Manufactured by Fisher Scientific Co., Chemical Manufacturing Division Fair Lawn, New Jersey 07410

Small pieces of biopsy material were used for electron microscopic study. Particular care and patience was exercised in orienting the small pieces during the embedding process with the aid of a dissection microscope. Single and multiple follicles were oriented for longitudinal and cross sections of the hair follicles. After cutting the small piece of skin into one-by-two millimeter rectangles, they were fixed for 2 to 3 hours in 3% glutaraldhyde (Sabatini, Bensch and Barrnett, 1963) buffered in phosphate and s-collidine (Bennett and Luft, 1959). Then the specimens were post fixed in 1% Osmium tetroxide buffered with phosphate or with s-collidine for 1-2 hours, (Millonig, 1961). They were then dehydrated through a graded series of alcohols and brought up to absolute alcohol and then processed through three changes of propylene oxide. The specimens were embedded in Epon 812 (Shell Chemical)[g] (Luft, 1961). Sections were cut at 1-3 u with the LKB8800A Ultratome III[h] and stained with toluidine blue (Cubberly, 1968) for light microscopy and for orientation for electron microscopic study. Ultrathin sections displaying gold and silver interference color were obtained by the use of a Reichert Ultramicrotome, using glass knives. Some of the sections were stained with lead citrate (Reynolds, 1963) followed by 1% uranyl acetate solution (Watson, 1958) and some were stained with only one of the stains. The sections were examined and photographed with an RCA 3H electron microscope at the Center for Electron Microscopy, University of Illinois.

Weekly hair samples were collected with the aid of a fine toothed comb, having 8 teeth per cm. This was done by combing the saddle region,

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[g]Shell Chemical Co., New York, New York

[h]LKB-Produkter AB S-161 25 Bramma 1 Sweden

ten strokes with the comb, after which the combed hair was gathered carefully and saved in envelopes with the name of the dog and the date written on the envelope. The combed hair samples were weighed on a Mettler H10T electric balance (sensitivity 1/1000 of mg.) and the results were recorded. Then the weight of the combed hair was totaled monthly for each dog and arranged on graphs.

In order to study hair growth rates, a method of study was tried that was described by Comben (1951). This consisted of shaving the area to be studied and reshaving various sections of this area at weekly intervals. The segments of hair shaft resulting from shaving were then washed to remove the soap scum and then filtered and allowed to dry on filter paper. The segments of hair were then measured by means of viewing them through a dissecting microscope with a calibrated scale in the field. There was extreme variation in length of the shaved whiskers. This variation resulted from the fact that the hair shafts of different hair follicles in a region are growing at different rates. With a mosaic arrangement, hairs in early anagen, late anagen, catagen, and telogen would be mixed together in the same region. The average of the length of all the cut whiskers that resulted from shaving after a specific period of growth would represent a mixture of almost no growth, slow growth and rapid growth. In an effort to determine the most rapid rate of growth a method was needed that would give figures for only the longest hairs that developed after previous clipping. These represented the anagen follicles. Consequently the method of Comben (1951) was discontinued and a new method was developed.

The method that was developed consisted of clipping the hair around the saddle region with an Oster small animal electric clipper, Model A2,

size 40 blade. After a week, ten of the longer hairs were measured with a ruler graduated in millimeters, and values were determined in a similar manner every week over a two month period for each dog. This was done by placing the end of the ruler in contact with the surface of the skin and directing it parallel to the direction of the hair. Then the measurements of the ten hairs were totaled and averaged as length per that period. The average length for each week was divided by seven resulting in the average rate of hair growth per day. The amount of hair growth and the rate of hair growth were plotted on graphs according to season of the year.

TABLE 1

Description of Beagle dogs according to their ages and sampling dates for biopsy collection

Name	Birth	Breed	Sex	9-72	10-72	11-72	12-72	1-73	2-73	3-73	4-73	5-73	6-73	7-73	8-73
ME-1	9-11-72	Beagle*		2W[†]	1M[○]	2M	3M	4M	5M	6M	7M	8M	9M	10M	11M
KV-2	9-11-72	Beagle*		2W	1M	2M	3M	4M	5M	6M	7M	8M	9M	10M	11M
LL-3	9-11-72	Beagle*		2W	1M	2M	3M	4M	5M	6M	7M	8M	9M	10M	11M
GM-4	9-10-71	Beagle		12M	13M	14M	15M	16M	17M	18M	19M	20M	21M	22M	23M
KF-5	9-10-71	Beagle		12M	13M	14M	15M	16M	17M	18M	19M	20M	21M	22M	23M
GR-6	9-10-71	Beagle		12M	13M	14M	15M	16M	17M	18M	19M	20M	21M	22M	23M
EN-7	12-14-70	Beagle		21M	22M	23M	24M	25M	26M	27M	28M	29M	30M	31M	32M
GH-8	12-14-70	Beagle		21M	22M	23M	24M	25M	26M	27M	28M	29M	30M	31M	Died
FL-9	12-14-70	Beagle		21M	22M	23M	24M	25M	26M	27M	28M	29M	30M	31M	32M

*Sampling of the dogs ME-1, KV-2 and LL-3 was conducted on the 15th day of each month except the September sample of 1972 which was collected on the 25th of September. The biopsy samples for all other dogs was collected during the last three days of each month.

†Weeks

○Months

FIGURE 1 Dog from Group 2

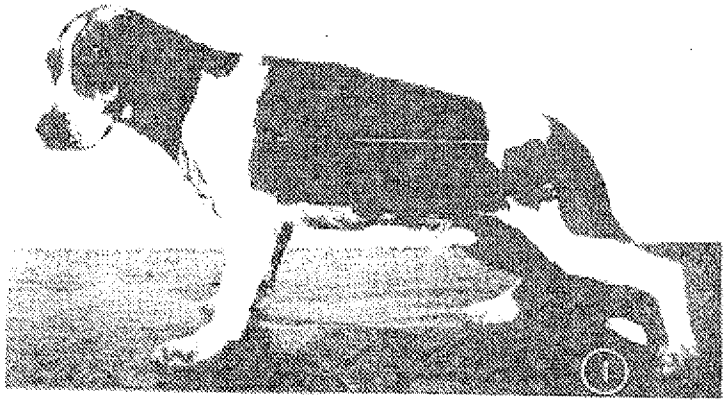
Representation of the type of dogs used in this study. One-year old male Beagle dog, KF-5.

FIGURE 2 Dog from Group 3

Dogs were selected that had extensive areas of white hair on the body surface. Two year old male Beagle dog, GK-9.

FIGURE 3 Biopsy Site

Clipped area in the saddle region prepared for a biopsy. Picture is from a six month old male Beagle dog, KV-2.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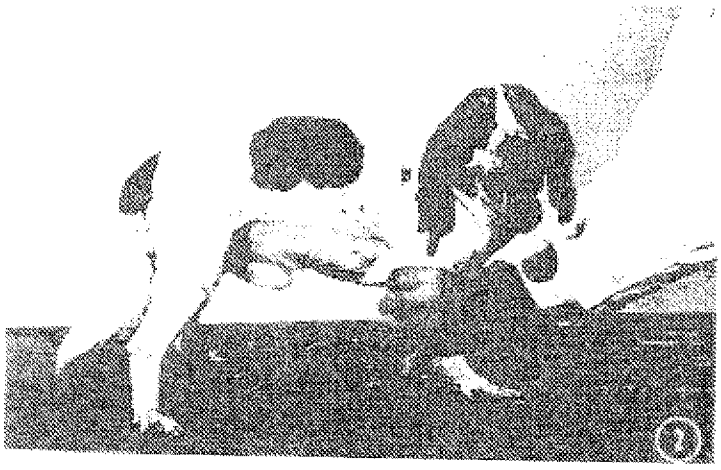
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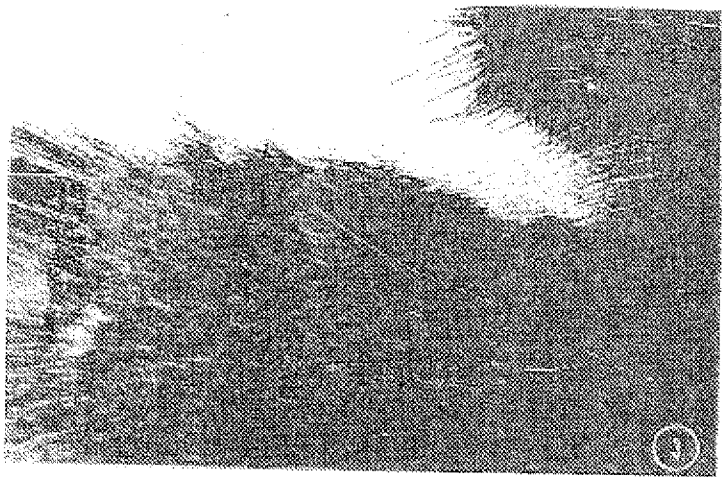
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FIGURE 4 Biopsy Gun

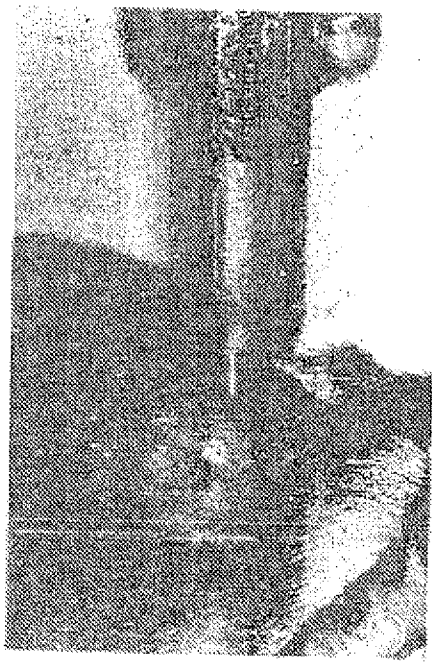
Area of skin in the saddle region prepared for collection of a biopsy. The biopsy gun is in position for collection of specimen. The hair was intentionally left 1/8 inch long so that the hair slant direction could be observed on biopsy specimens.

FIGURE 5 Biopsy Wound

A circular wound is left after the biopsy is collected. Part of the subcutaneous adipose tissue can be observed in the wound.

FIGURE 6 Appearance Following Closure of Wound

Four 20 mm. wound clips were used to close the biopsy opening.



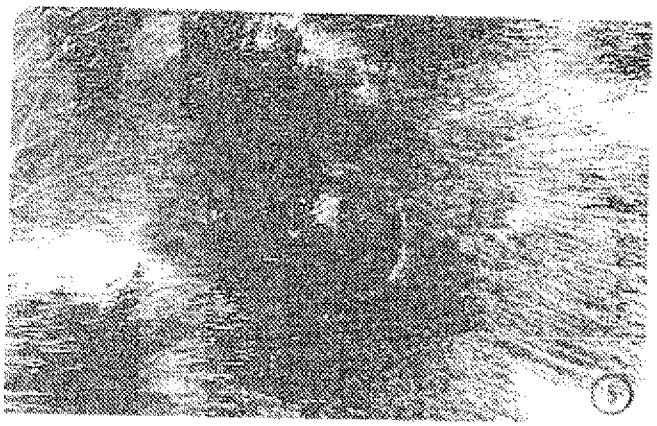


FIGURE 7 Biopsy Incision

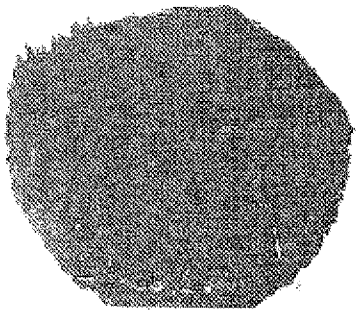
Saddle region of a male Beagle dog with biopsy before removal. The biopsy gun creates a circular incision by rotation. Subcutaneous tissue must be cut to remove the biopsy specimen.

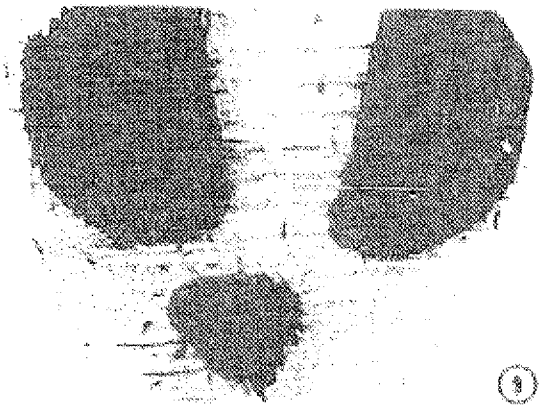
FIGURE 8 Biopsy Specimen

Circular shaped biopsy specimen showing the direction of slant of the hairs.

FIGURE 9 Division of Sample

Biopsies were divided into two halves and a small piece was removed from one half. This small piece was used for electron microscopy. One large piece was used for histochemistry while the other was used for hematoxylin and eosin histological studies.





IV. RESULTS

A. General Definition of the Hair Cycle Stages in the Dog

Examination of the biopsies collected, revealed that the hair coat in the dogs studied is made up of compound hair follicles. They consist of main hair follicles associated with a group of secondary hair follicles (Figs. 10 and 11). These compound hair follicles are slanted in relation to the surface of the skin (Fig. 10). The secondary and the main hair follicles extend to different depths into the dermis (Fig. 11). The larger hairs tend to extend more deeply than smaller hairs. All the hair follicles that compose a compound hair follicle tend to be parallel to each other in the dermis (Figs. 10 and 11). The component follicles making up a compound follicle are not necessarily in the same stage of cyclic activity and may be in distinctly different stages. In the biopsy material of this study, it was possible to identify the various stages of the hair cycle that were described by Dry (1926): anagen (active), catagen (transitional), telogen (resting). The anagen stage was found to be characterized by a well developed spindle shaped dermal papilla. This dermal papilla is completely capped by the hair matrix cells which together with the dermal papilla make up the bulb of the hair follicle (Fig. 12). At low magnification the bulb appears as an enlargement at the terminal end of the follicle. Catagen stage is identified by a thick glassy membrane on the outside of the follicle (Fig. 13). This glassy membrane is irregular and undulating at the lower third of the hair follicle. Thickening of the glassy membrane is accompanied by a thickening of the basement membrane between the dermal papilla and the matrix. The bulb of the hair follicle during catagen becomes smaller and the dermal papilla becomes more rounded rather than spindle shaped (Fig. 13). Hair follicles during telogen stage have a

smaller dermal papilla than was observed in anagen and catagen (Fig. 14). The dermal papilla during telogen is separated from the bulb and is no longer capped by matrix cells which have decreased in number (Fig. 14). The hair follicle of telogen contains a club hair and the inner root sheath disappears (Fig. 14).

B. Percentage of Follicles in Hair Cycle Stages Observed in Histological Sections of Biopsies

Based upon the distinguishing morphological features of the three hair follicle stages, counts were carried out by means of light microscope observation as described in the Materials and Methods. The counts and percentages of hair follicle stages are presented for the 9 dogs studied on Table 2. The percentages are more easily analysed by observing graphs indicating the percentages of the follicle stages month by month for the entire period of study. Figure 15 is a graphic presentation of the findings for the three dogs studied during the first year of life. Figure 16 is a presentation of the mean values of all three of the dogs on one graph. It can be readily observed that there is a marked similarity between the curves plotted for these three dogs. Consequently, the graph generated from the mean of all three dogs indicates peaks of anagen and telogen at approximately the same month or season of the year as it did for the individual dogs.

Figure 17 is a graphic presentation of the findings for the three dogs studied during the second year of life and Figure 18 represents the mean of all three dogs on one graph.

Figure 19 is a graphic presentation of the findings for the three dogs studied during the third year of life and Figure 20 represents the mean of all three dogs on one graph.

As was true of the dogs studied during the first year of life there is a similarity between the graphs prepared for individual dogs during the second and third year of life and the graphs representing the mean values for the second and third year dogs.

Figure 31 represents the average monthly temperatures during the time the study was conducted. By comparing this temperature graph with those of the individual or mean percentage of hair follicle stage graphs it can be noted that there are generally two anagen peaks during the year. One tends to occur during the colder months of the year, and the other occurs during the warmer months of the year. Some variation was noted (Fig. 17) but the peaks mainly occurred in the summer and winter. It can be further noted that two peaks occur for telogen in each graph. One in the fall during a transitional temperature period of warm to cold, and one in the spring during a transitional temperature period of cold to warm. The percentages for catagen are low ranging from 0% to 7%. The graphic representation for catagen does not produce peaks as there are for anagen and telogen. Because the catagen percentage is low and fairly uniform, the curves for anagen and telogen are almost mirror images of one another. When anagen is high telogen is the lowest when anagen is minimal, telogen is at its highest level.

With one exception telogen percentage is always lower than anagen percentage. Telogen ranged from a low of around 8% to a high of 53%. In one instance, telogen exceeded 50%. Anagen ranged from a low of 47% to a high of 92%. In the group of 1 year old dogs telogen values tended to be about 10% lower and anagen was about 10% higher than in the older dogs.

Figure 21 represents the mean percentage values of each of the three age groups placed end to end giving a time sequence perspective to the percentages.

The lower telogen percentages for dogs during the first year of life is evident on this graph. It is also interesting to note that 12% of the follicles observed in puppies two weeks of age were in telogen.

C. Seasonal Changes in Hair Shedding as Studied by Collection of Combed Samples of Hair

The hair in the saddle region of seven dogs was combed each week by the technique described in the Materials and Methods. Table 3 presents a summary of the values determined for each dog when the combed hair samples were weighed.

Figure 22 is a graphic presentation of the material collected from the three dogs that were studied from 2 months to 15 months of age. Figure 23 is a similar presentation for the three dogs studied from 21 months to 36 months of age. Because of the fact that one dog in the group studied from 12 to 24 months of age was clipped all over in order to study the patterns of hair regrowth, this dog was not included in the combing sampling. Dog CR-6 was nervous and bit several attendants during handling. This dog was not included in the combing sampling because of the handling difficulties. Consequently, combed samples were collected only from Dog KF-5 in the 2 year group. Table 4 represents the mean values for each of the three groups of dogs that were used in plotting the graph in Figures 24, 25 and 26.

Figure 24 is a graph representing the mean of the values obtained for the weight of the combed hair for the group studied during the first year of life, the group studied during the third year of life together with the values from the one dog studied during the second year of life.

Figure 25 is a graph which combines the 3 graphs from Fig. 24 in sequence compared with the mean temperature curve. It can be noted that shedding tended to occur in the spring and fall seasons.

Figure 26 is a combination graph of Figure 25 together with a three year seasonal mean percentage of telogen counts taken from Figure 21. It can be noted that with two exceptions telogen peaks and shedding peaks occur approximately at the same time. In one case the shedding peak occurred at the time telogen was increasing, in the other the shedding peak occurred when telogen was at its lowest point.

D. Growth Rate of Hair Shaft

An area of the saddle region of six dogs was clipped once in the summer and once in the winter in order to study the growth rate of the hair shaft. The method was carried out according to the description in the Materials and Methods. Two dogs were studied in this manner in each of the three age groups. The values determined for each dog are presented on Table 5. The means for the measurements performed on the two dogs in each group were determined and are presented on the three graphs included in Fig. 27. The mean weekly hair lengths for all 6 dogs during the 8 weeks studied are presented on Fig. 28. At 1 week following clipping the average hair length was in the order of 3 mm. After 8 weeks after clipping it was 16 to 19 mm.

The mean daily rate of hair growth for two dogs in each age group was determined according to the method described earlier (page 35). This data is presented in Fig. 29. The values vary between .30 and .55 mm. of growth per day.

Figure 30 is a graphic representation of the mean daily hair growth rate values for all 6 dogs. The growth rate in winter varied from .33 mm. to .54 mm. per day and for summer it varied from .27 mm. to .42 mm. per day. The overall variation of hair growth rate was from .27 mm. to .54 mm. per day.

Figure 31 is a graph of the average temperature during the period of study recorded at the Urbana Weather Station at Morrow Plots. This was 2 blocks from the Old Small Animal Clinic where the experimental animals were housed and exposed to outside temperatures for 7 hours each day.

E. Detailed Histological and Histochemical Observations of the Hair Cycle Stages

A brief description and definition of the hair cycle stages was presented in section A to identify the morphological basis on which the counts and percentages were determined for the charts and graphs. Detailed observations were made in addition to those already described. These will now be presented for each stage of the hair follicle cycle.

1. Anagen

The hair follicle in anagen stage extends deep into the dermis (Fig. 32). Its bulb extends into the adipose tissue. The line of demarcation between the dermis and the subcutaneous tissue is irregular. The bulbs of the larger follicles of the main hair extend into the subcutaneous fat (Fig. 10). The bulbs of the smaller secondary follicles are also surrounded by fat cells because tracts of fatty tissue extend up into the dermis around the follicle complexes. Consequently the lower 1/3 of the follicles are surrounded by fat. There is a thin layer of connective tissue between the bulb of the hair follicles and the adipose tissue. The connective tissue of the fibrous covering of the follicles is continuous with the dermal papilla at the base of the hair follicle bulb (Fig. 32). The connective tissue of the dermal papilla is surrounded by the matrix portion of the bulb. The upper tip of the flame shaped dermal papilla extends up from the bulb portion of the follicle into the narrower part

of the follicle into the region where the hair shaft is beginning to form (Fig. 32).

Following the technique for the detection of the presence of alkaline phosphatase the cells of the dermal papilla of anagen reacted in various ways. In the majority of anagen follicles observed, the dermal papilla reacted positively. Figure 33 illustrates a typical example of the reaction observed which is a generalized reaction in the entire dermal papilla area. In some instances the general reaction was quite weak and the cells of the capillary endothelium within the dermal papilla reacted strongly (Fig. 34). The reaction in some dermal papillae was strong in both the dermal papilla cells and the endothelial lining of blood vessels (Fig. 35). Figure 36 illustrates a similar reaction for alkaline phosphatase in a pigmented and a non-pigmented hair follicle. Figure 37 is an example of two anagen hair follicles one of which reacts strongly for alkaline phosphatase and the other reacts very weakly or negatively.

The dermal papilla generally did not contain glycogen granules except in a few instances and these were in very young dogs.

The basal cells of the matrix of the hair bulb line the dermal papilla area. The matrix cells that line the lower part of the basement membrane are columnar in shape (Fig. 32). The columnar cells over the tip of the dermal papilla appear to merge with the cells of the medulla of the developing hair shaft (Fig. 32). They are distinguishalbe from the rest of the matrix cells by their columnar shape and by their close proximity to each other. The matrix cells in the upper half of the hair follicle bulb appear to be ungergoing differentiation into medullary, cortical or inner root sheath cells. The matrix cells in the lower half of the bulb appear to be undifferentiated (Fig. 32). In pigmented hair follicles, melanocytes

appear between the matrix cells and melanin granules can also be observed within the cytoplasm of melanocytes and matrix cells.

Histochemically the cells of the matrix region of the hair bulb are inactive. Sometimes the nuclei of the matrix cells become darkened after treatment for the demonstration of alkaline phosphatase (Fig. 33). This only occurs when there is a very intense reaction for alkaline phosphatase in the dermal papilla and is interpreted as a diffusion artifact. The matrix cells were also negative for glycogen.

With hematoxylin and eosin staining, the glassy membrane surrounding the outer root sheath of the middle and lower 1/3 of the hair follicle appears as an indistinct layer. After PAS treatment the glassy membrane is a distinct pink presumably due to staining of the mucopolysaccharide content. The cells of the middle third of the outer root sheath appear to be large and vacuolated. They do not react to the treatment for the demonstration of alkaline phosphatase. Following PAS treatment, the cells of the outer root sheath of the middle third of the hair follicle were found to contain high quantities of dark purple granules which were interpreted as glycogen (Fig. 38). This interpretation was based on the fact that they disappear after treatment with diatase.

The layers of the inner root sheath can not be distinguished clearly with the hematoxylin and eosin staining. Histochemical studies produced the results that the inner root sheath is not reactive for glycogen or mucopolysaccharide. The inner root sheath does become darkened after treatment for the demonstration of alkaline phosphatase. This is not labeled but can be observed in Fig. 50. This is probably due to native phosphate content and occurs in both controls and specimens incubated in substrate containing sodium glycerophosphate.

The cortex and medulla of the hair shaft are discernable after H & E staining. In pigmented follicles, melanin granules can be observed in the cortex in great numbers (Fig. 36). A gradual transition in the morphology of the cells of the matrix can be observed as they ascend up into the hair shaft through the keratogenous zone and become completely keratinized. The cortex beyond the keratogenous zone has a yellow appearance in unpigmented hair shafts.

2. Catagen

During catagen stage the hair follicles do not extend as deeply into the dermis and subcutaneous tissue as they do during anagen. They are, however, still surrounded by adipose tissue (Fig. 39). The connective tissue sheath surrounding the lower third of the catagen follicle is similar in thickness to that observed during anagen stage. The unique characteristic of catagen stage is the thick glassy membrane which separates the connective tissue sheath of the lower third of the follicle from the outer root sheath epithelium (Fig. 39). In the middle third of the follicle the outer root sheath is thicker and contains many glycogen granules (Fig. 41). In late catagen the thickened glassy membrane becomes distinctly undulating in appearance above the bulb of the follicle. This can be observed in Fig. 39. Another feature of catagen is that the dermal papilla becomes rounded and the nuclei of its cells appear to stain with hematoxylin more distinctly than in anagen (Fig. 39). This darker appearance of the dermal papilla may be due to a lesser amount of cytoplasm in these cells allowing the nuclei to become more closely associated with one another producing a more compact appearance than was observed during anagen. In catagen the dermal papilla becomes round rather than tear drop or spindle shaped as was observed in anagen. The basement membrane separating the dermal papilla from the matrix

cells becomes more distinct especially after PAS treatment. This can be observed in Figure 41. The matrix cells during catagen appear to lose their orientation with the dermal papilla (Fig. 39). There are no longer any columnar cells lining the dermal papilla and the matrix cells all seem to become similar in appearance and seperate from the beginning club hair which becomes enlarged and keratinized. During catagen the dermal papilla does not contain glycogen but is strongly alkaline phosphatase positive (Fig. 40).

3. Telogen

During telogen stage the hair follicles do not extend as deeply into the dermis as they do during catagen. They are almost pulled up out of the tracts of adipose tissue. The tip of the follicle may be associated with a few fat cells.

Telogen stage is characterized by the fact that the dermal papilla is no longer surrounded by or closely associated with the matrix cells. The dermal papilla of telogen has become separated from the matrix cells and is completely surrounded by its own basement membrane (Figs. 42 and 43). During early telogen the dermal papilla appears as an inverted cap which shares a common basement membrane with the matrix cells (Fig. 42). In late telogen the dermal papilla is spherical and its basement membrane contacts the basement membrane of the bulb of the follicle only at one point (Fig. 43). The dermal papilla was found to be strongly alkaline phosphatase positive as observed in Figures 45 and 46. The reaction is not confined to the blood vessels but is evident in all parts of the dermal papilla.

The remaining cells, representing the matrix, are reduced in number and some are destined to become the hair germ (Fig. 43), which presumably will become active again when the follicle goes into anagen. The cells all appear to be uniform in their appearance and form a cluster of cells at the

bottom of the epithelial part of the follicle (Fig. 43). They are continuous with the cells of the outer root sheath which are negative for glycogen even up into the middle third of the follicle (Fig. 42). The glassy membrane is PAS positive and can be observed as a thin pink uniform structure between the connective tissue surrounding the follicle and the outer root sheath (Fig. 42). The glassy membrane of telogen in the deep 1/3 of the follicle is much thinner than the glassy membrane of catagen but it is thicker than the glassy membrane of anagen (Fig. 43). A distinguishing feature of telogen is the club hair which has acquired a brush-like appearance (Figs. 43 and 44). Strands of keratin can be observed with the light microscope penetrating between the cells of the outer root sheath and the remnants of the matrix cells which are located below the club hair (Fig. 43). During telogen there is no inner root sheath of the follicle present (Fig. 44).

F. Relationship Between the Main Hair Follicles and the Secondary Hair Follicles

It was observed in the course of this work that the outer root sheaths of the secondary hair follicles join the outer root sheath of the main hair follicle at the level of the sebaceous gland. Above this point all the hairs of a group of main and secondary hairs share a common neck of the follicle between the sebaceous gland and the surface of the epidermis. The epithelium of this common neck of the follicle complex is similar in morphology and continuous with the surface epithelium of the epidermis. This arrangement is illustrated in Figure 47 in which one can observe the confluence of three secondary follicles with one main hair follicle. The fundus of the sebaceous glands are evident at this level. Figure 48 is a higher power view of the same area showing this arrangement.

G. Appearance of Secondary Hair Follicles and Telogen Follicles at Two Weeks of Age

Histological sections of biopsies cut parallel to the surface of the skin were observed in this study. It was observed that as many as 6 or 9 secondary follicles were associated with each main hair in the specimens collected at 2 weeks of age. This is illustrated in Figure 49.

It was also observed that telogen follicles were regularly present in the biopsies collected at 2 weeks of age from all three puppies in the group. Twelve percent of the follicles evaluated at this age were in the telogen stage (Fig. 15). Figure 50 illustrates a telogen hair follicle following treatment for the demonstration of alkaline phosphatase. The dermal papilla of this follicle is dark indicating a positive reaction for the enzyme. A club hair shaft can also be observed in the smaller secondary hair follicle.

H. Cellular Details Observed in Anagen and Telogen by Means of the Transmission Electron Microscope.

1. Anagen

The cells of the dermal papilla during anagen stage appear to be spindle shaped with processes which on cross section can be observed to contain organelles similar to what is observed within the main body of cytoplasm around the nucleus (Fig. 51). The nuclei of the cells of the dermal papilla have a more regular distribution of chromatin than the nuclei of the matrix cells (Fig. 52). The zone between the dermal papilla cells and the matrix cells was observed to occur in two different types of arrangement. Figure 52 illustrates one type in which this area is occupied by cytoplasmic processes. Figure 53 illustrates another type in which the cytoplasmic processes are not evident and the area is occupied by granular

ground substance and electron lucent circular areas. On Figure 53 it can be observed that there are areas of similar density within the cytoplasm of the dermal papilla cells that border the area.

The cells of the matrix of the hair bulb have nuclei with areas of clumped chromatin (Fig. 52). The columnar cells of the matrix along the basement membrane of the dermal papilla have extensive amounts of cytoplasm between the nucleus and the basal border of the cell. Mitochondria can be observed in their cytoplasm.

Figure 54 illustrates the morphology of the cells of the upper part of the bulb of the anagen hair follicle. The cells at the periphery are differentiating into outer root sheath cells. A definite basement membrane can be observed between the outer cells and the fibrous connective tissue of the connective tissue sheath of the follicle. The basement membrane is not very distinct and blends with the glassy membrane which appears as a laminated zone of ground substance which increases in density on the outer margin. Outside of this there are many collagen fibers that appear to be arranged in several layers which are oriented in different directions. It can be observed in Figure 54 that the chromatin of the nucleus of the matrix cells is clumped and unevenly distributed. Small desmosome attachments can be observed between the plasma membranes of adjacent cells. Mitochondria and endoplasmic reticulum can be observed in the cytoplasm of the matrix cells. Cell processes of melanocytes, or melanocytic dendrites, or cytoplasm of matrix cells containing melanin granules can be observed in the lower half of the picture.

In heavily pigmented hair follicles the matrix area has many melanin containing cells. Figure 55 illustrates a cell from this region in which melanin accumulations of various sizes can be observed. The Golgi

apparatus can be observed near small masses of developing melanin. Because of the varying developmental stages of melanin observed in this cell it was interpreted as a melanocyte.

Figure 56 represents the arrangement of the cells in the middle region of the lower third of the hair follicle (just above the bulb). In the upper right hand corner of the picture the basement membrane appears as a dark zone along the cell boundry. Outside of this the glassy membrane can be observed. This is re-enforced by collagen fibers that are farther out. The cells of the outer root sheath are arranged in a layer 2 or 3 cells thick. The outstanding feature of these cells is the presence of large electron lucent membrane bound vesicles and glycogen granules in the cytoplasm. In some instances the glycogen granules surround the vesicles. The cytoplasmic margin of the outer root sheath cells bordering Henle's layer contains many small irregular granules. Because this specimen was taken from a level close to the bulb of the follicle, the structures of the inner root sheath and the cortex and cuticle of the hair shaft have not yet completely developed. Henle's layer is represented by a band of keratinized material which represents a cell on the left side of the picture and by large trichohyalin granules and smaller fibrous material in another cell on the right side of the picture. Huxley's layer is represented by a layer of cells containing small numbers of small granules. The cells that will form the cuticle of the inner root sheath cannot be discerned. Several layers of cells destined to become the cuticle of the hair shaft can be observed. The cell membranes are distinct and degenerating nuclei can be observed in some cells. The cells are flattened somewhat and evidence of cytoplasmic degeneration can be observed. Beginning stages of keratinization can be observed in the cuticle cells. The plasma membranes

of the developing cuticle cells are distinct. In the lower left corner, a small part of the cortex of the hair shaft can be observed containing numerous keratin fibers and melanin granules.

Figure 57 represents the arrangement of the cells in the hair follicle wall at the level of the junction of the bottom 1/3 and the middle 1/3 of the hair follicle of anagen. A small portion of the outer root sheath may be observed in the upper right hand corner. At this level the various layers of the inner root sheath are more distinct. Henle's layer appears as an almost homogeneous band of keratinized material except at the intercellular junction. Huxley's layer contains trichohyalin granules of various sizes. The cells destined to become the cuticle of the inner root sheath are flattened and contain trichohyalin granules which are smaller than those in Huxley's layer. The cells that will become the cuticle of the hair shaft are greatly flattened and keratinized. The plasma membrane may be observed in some locations. The outer side of these flattened cells is filled with small dense cross sections of keratin bars. The other side is filled with a less dense granulated cytoplasm which contains a few fibrous elements. The cortex of the hair shaft fills the lower half of the picture. There is an abundance of cross sections of large keratin cortical fibers and sparcely distributed melanin granules, the remaining space is occupied by granular cytoplasm. Occasionally a nucleus is observed. Cell membranes cannot be discerned.

2. Telogen

The cells of the dermal papilla during telogen stage appear to have larger nuclei and less cytoplasm than the same cells during anagen (Fig. 58). Organelles are present but not as numerous as during anagen. There are small desmosome attachments of the plasma membranes at infrequent points

along the line of the cell boundary between adjacent cells. In general the dermal papilla cells of telogen appear to be more spherical possessing fewer spindle shaped processes than those of anagen. There is variation in the shape and density of the telogen dermal papilla cells. The darker cells are more irregular in shape than the lighter cells. The basement membrane surrounding the dermal papilla is covered by spindle shaped fibroblasts.

Figure 59 illustrates the appearance of the relationship between the cells of the outer root sheath and the club hair in the lower 1/3 of the telogen hair follicle. Large desmosomes are evident between adjacent cells of the outer root sheath which have thick bundles of fibrils attached to them. Strands of keratinized cortical material are evident in invaginated folds of the cytoplasm of the outer root sheath cells.

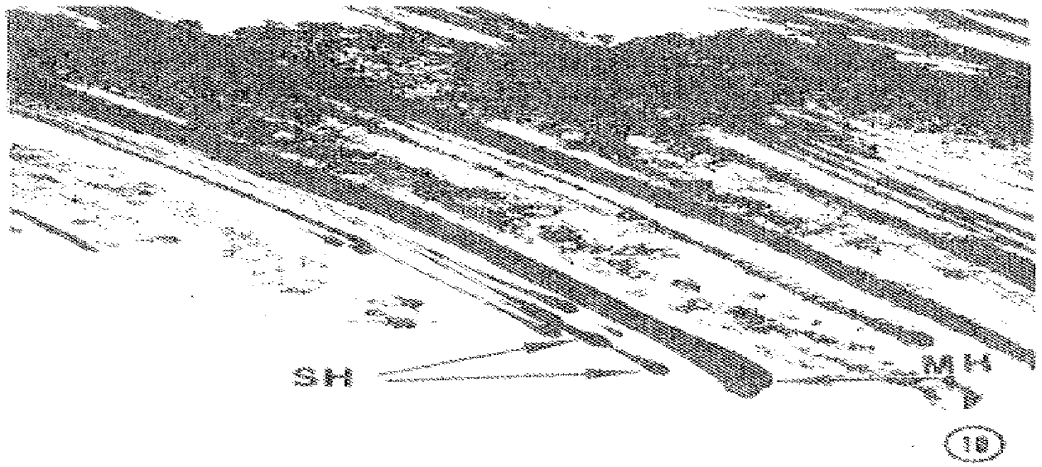
The region of the junction of the connective tissue covering of the middle 1/3 of a telogen follicle with the epithelium of the outer root sheath may be observed in Figure 60. Here the desmosomes forming attachments between the plasma membranes of adjacent cells may be observed. Mitochondria can be observed in the cytoplasm as well as bundles of filaments. There is an absence of vesicles or glycogen granules as opposed to what was observed during anagen. The basement membrane of the outer root sheath is re-enforced by collagen fibers some of which appear to attach to the basement membrane or the plasma membrane of the outer root sheath cells. Outside of this, some cytoplasmic processes of fibroblasts which are associated with the connective tissue covering the hair follicle can be seen.

FIGURE 10 Compound Hair Follicle

Two hundred micron thick section of skin from a thirteen month old Beagle dog. The specimen is from the saddle region of dog LL-3. The compound hair follicles, consist of a main hair follicle (MH) and secondary hair follicle (SH). Unstained section. Magnification 70X.

FIGURE 11 Compound Hair Follicle

Higher magnification of the same specimen as seen in Figure 10. The bulbs of the follicles making up the complex extend to different depths into the dermis. The main hair (MH) extends deeper into the adipose tissue than the secondary hair follicles (SH). The main and the secondary hair follicles are parallel to each other in the dermis. Unstained section. Magnification 175X.



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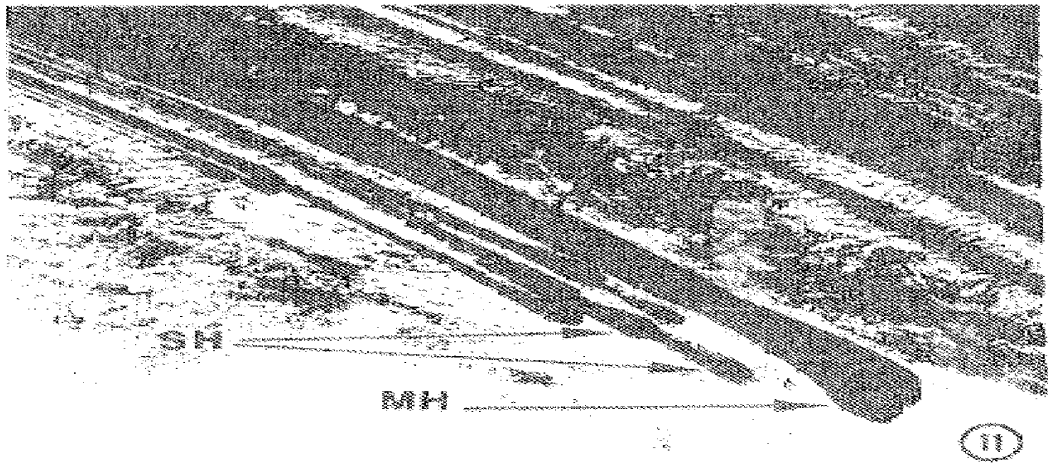


(11)

FIGURE 12 Anagen

Longitudinal section of a main hair follicle from the saddle region of a six month old Beagle dog. This is an example of anagen stage and it illustrates a well developed dermal papilla (DP), which is completely bordered by the matrix cells (Mx). The bulb of the hair follicle is labeled (Bb). Magnification 240X. Stained with hematoxylin and eosin, from dog KY-2.

FIGURE 13 Catagen

Longitudinal section of a main hair follicle from the saddle region of a two week old Beagle dog. This is an example of a catagen hair follicle. It has a rounded dermal papilla (DP). The glassy membrane (GM) is thick and somewhat irregular above the bulb region. The basement membrane (BM) can be observed. Magnification 265X. Stained with hematoxylin and eosin, from dog ME-1.

FIGURE 14 Telogen

Longitudinal section of a main hair follicle from a 9 month old Beagle dog. This is an example of a telogen hair follicle. The dermal papilla (DP) is outside of the bulb, separated from the matrix cells by a basement membrane (BM). The outer root sheath (ORS) borders the club hair (CH) directly due to lack of inner root sheath. Magnification 190X. Stained with hematoxylin and eosin, from dog KY-2.

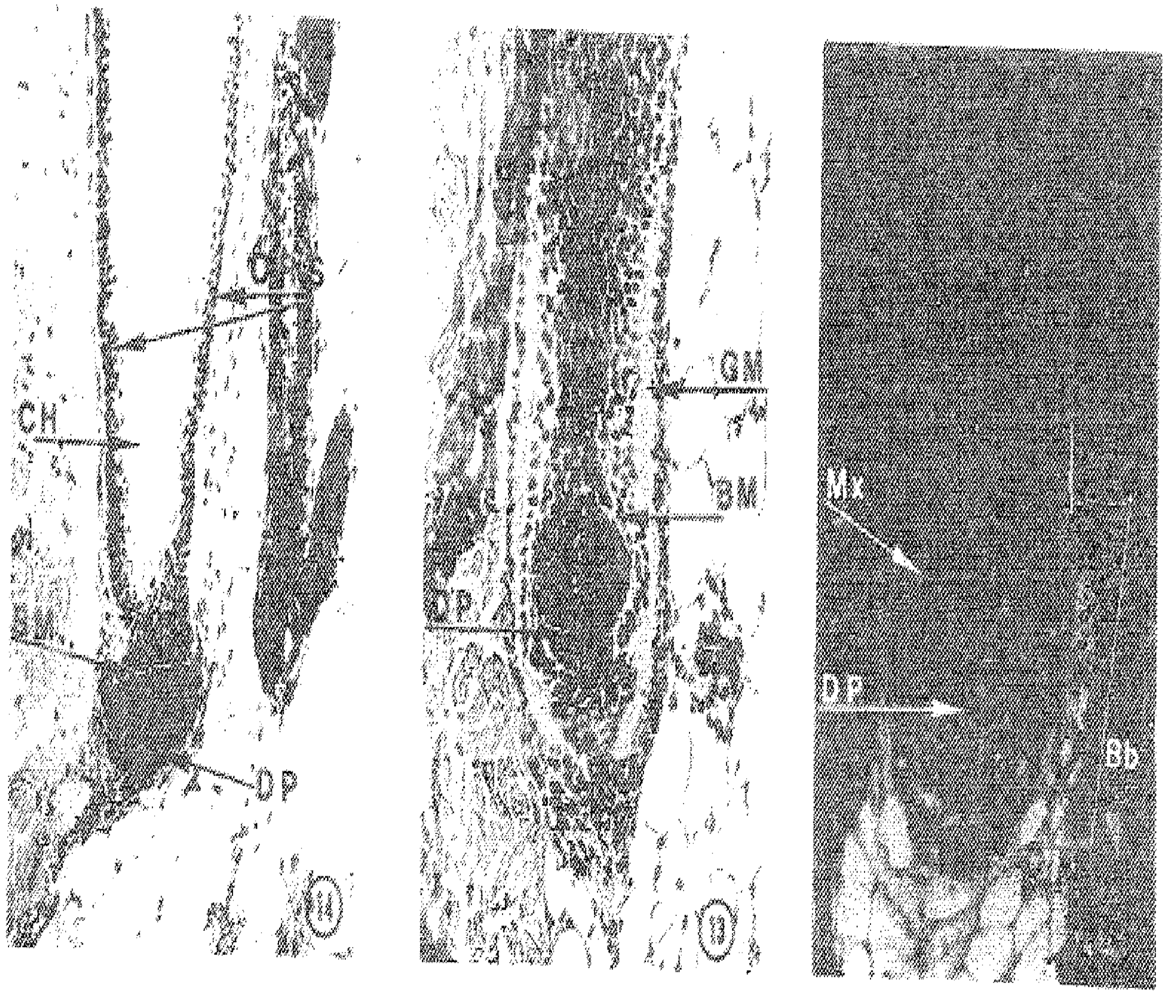

CH
DP
14
GM
BM
DP
13
Mx
DP
Bb

TABLE 2

Light Microscopic Counts of the Hair Cycles during One Year in Nine Beagle Dogs

Name	Sept		Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		June		July		Aug	
Age HE-1	2 W		1 M		2 M		3 M		4 M		5 M		6 M		7 M		8 M		9 M		10 M		11 M	
		%		%		%		%		%		%		%		%		%		%		%		%
Anagen	158	87	16	57	257	78	453	92	205	88	200	87	278	71	136	58	186	90	179	74	203	80	207	88
Catagen	2	1	2	7	5	2	0	0	5	2	4	1	3	1	2	1	0	0	2	1	12	5	0	0
Telogen	22	12	10	36	66	20	38	8	21	10	41	12	105	28	99	41	119	40	62	25	38	15	27	12
Total	182		28		328		491		231		335		378		237		309		243		244		234	
Age KV-2	2 W		1 M		2 M		3 M		4 M		5 M		6 M		7 M		8 M		9 M		10 M		11 M	
		%		%		%		%		%		%		%		%		%		%		%		%
Anagen	118	88	113	86	130	63	132	76	295	86	198	87	196	78	119	65	126	63	193	82	163	87	190	79
Catagen	0	0	2	1	0	0	0	0	0	0	2	1	0	0	0	0	2	1	0	0	3	1	11	5
Telogen	16	12	17	13	80	37	42	24	49	14	26	12	56	22	98	46	73	36	43	18	22	12	39	16
Total	134		132		210		174		344		216		252		217		201		236		188		240	
Age LL-3	2 M		1 M		2 M		3 M		4 M		5 M		6 M		7 M		8 M		9 M		10 M		11 M	
		%		%		%		%		%		%		%		%		%		%		%		%
Anagen	70	84	70	64	41	63	130	78	78	82	70	76	72	64	119	54	130	63	114	77	140	79	180	84
Catagen	0	0	0	0	3	7	4	2	1	1	2	2	8	7	5	2	3	1	0	0	0	0	3	1
Telogen	13	16	39	36	19	30	32	20	16	17	20	22	32	29	97	44	74	36	33	23	40	21	30	15
Total	83		109		65		166		95		92		112		221		207		147		188		213	

W - Weeks of age at time of biopsy.

M - Months of age at time of biopsy.

TABLE 2 (cont'd)

Light Microscopic Counts of the Hair Cycles during One Year in Nine Beagle Dogs

Name	Sept		Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		June		July		Aug	
Age CM-4	12 M		13 M		14 M		15 M		16 M		17 M		18 M		19 M		20 M		21 M		22 M		23 M	
		%		%		%		%		%		%		%		%		%		%		%		%
Anagen	94	74	109	64	197	54	101	47	109	53	158	69	146	77	355	75	167	59	191	57	245	68	265	72
Catagen	3	2	2	1	8	2	0	0	0	0	0	0	1	1	8	2	0	0	0	0	0	0	3	1
Telogen	29	24	57	35	158	44	113	53	95	47	71	31	42	22	110	23	114	41	142	43	115	32	117	27
Total	126		169		363		214		204		229		189		473		281		333		360		395	
Age KF-5	12 M		13 M		14 M		15 M		16 M		17 M		18 M		19 M		20 M		21 M		22 M		23 M	
		%		%		%		%		%		%		%		%		%		%		%		%
Anagen	196	79	196	77	262	63	152	55	215	79	346	76	312	70	146	65	204	55	226	67	204	75	238	70
Catagen	2	1	2	1	12	3	7	3	0	0	4	1	1	0	4	2	5	1	6	2	0	0	7	2
Telogen	49	20	56	22	142	34	118	42	59	21	103	23	130	30	72	32	165	44	105	31	67	25	95	28
Total	247		254		415		277		275		453		443		222		374		339		271		340	
Age CR-6	12 M		13 M		14 M		15 M		16 M		17 M		18 M		19 M		20 M		21 M		22 M		23 M	
		%		%		%		%		%		%		%		%		%		%		%		%
Anagen	130	77	140	69	232	55	208	79	336	75	277	76	180	68	199	63	201	54	229	71	279	75	260	72
Catagen	4	2	1	0	6	1	2	1	4	1	4	1	0	0	2	1	4	1	2	1	6	2	5	1
Telogen	34	21	56	31	182	44	53	20	109	24	104	29	84	32	117	36	169	45	93	28	87	23	95	27
Total	168		204		420		263		448		365		264		318		374		324		372		360	

M - Months of age at time of biopsy.

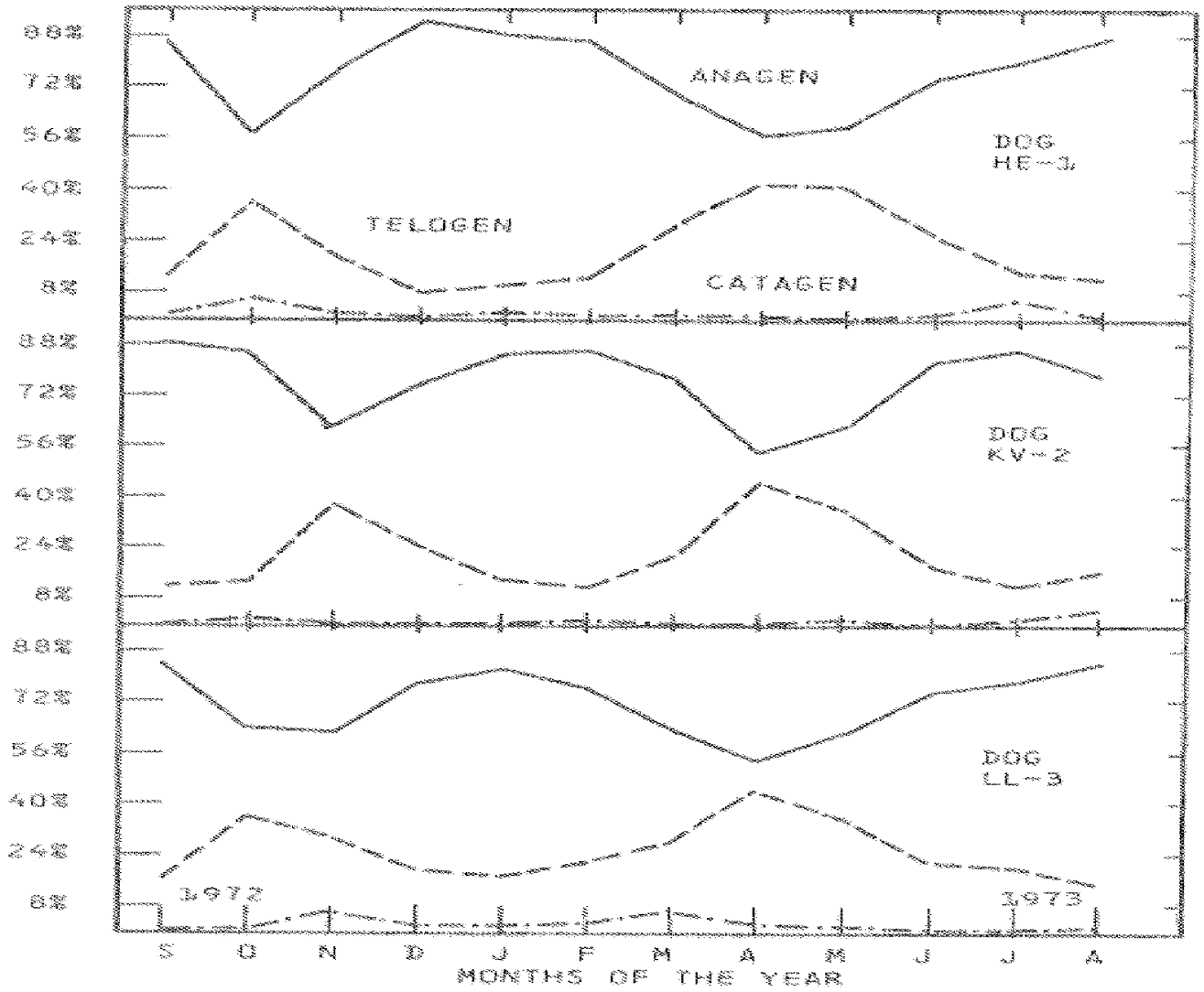
TABLE 2 (cont'd)

Light Microscopic Counts of the Hair Cycles during One Year in Nine Beagle Dogs

Name	Sept		Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		June		July		Aug	
Age	21 M		22 M		23 M		24 M		25 M		26 M		27 M		28 M		29 M		30 M		31 M		32 M	
Ell-7		%		%		%		%		%		%		%		%		%		%		%		%
Anagen	148	67	182	64	127	50	126	70	107	72	152	78	96	60	206	51	136	54	382	71	301	73	265	70
Catagen	1	0	1	0	7	3	0	0	0	0	0	0	0	0	8	2	5	2	5	1	0	0	5	1
Telogen	73	33	103	36	120	47	54	30	41	28	34	22	65	40	186	47	110	44	148	28	105	27	109	29
Total	222		286		254		180		148		195		161		400		251		535		407		376	
Age	21 M		22 M		23 M		24 M		25 M		26 M		27 M		28 M		29 M		30 M		31 M		32 M	
GH-8		%		%		%		%		%		%		%		%		%		%		%		%
Anagen	222	70	214	56	172	55	316	77	280	80	239	72	242	53	283	57	148	53	231	69	327	74	Died	
Catagen	3	1	3	1	0	0	3	1	2	1	2	1	0	0	4	1	2	1	6	2	0	0		
Telogen	93	29	163	43	139	45	92	22	68	19	92	27	209	47	148	42	127	46	100	29	112	26		
Total	318		380		311		411		350		333		451		355		277		337		439			
Age	21 M		22 M		23 M		24 M		25 M		26 M		27 M		28 M		29 M		30 M		31 M		32 M	
FL-9		%		%		%		%		%		%		%		%		%		%		%		%
Anagen	98	71	221	66	154	53	227	70	233	76	159	79	224	69	267	56	303	59	324	70	350	72	283	73
Catagen	1	1	7	2	4	1	6	2	3	1	0	0	1	0	4	1	4	1	6	1	3	1	2	1
Telogen	39	28	156	33	131	46	93	28	71	23	43	21	155	41	203	43	209	40	133	29	132	27	105	26
Total	138		484		289		326		307		202		380		474		516		463		485		390	

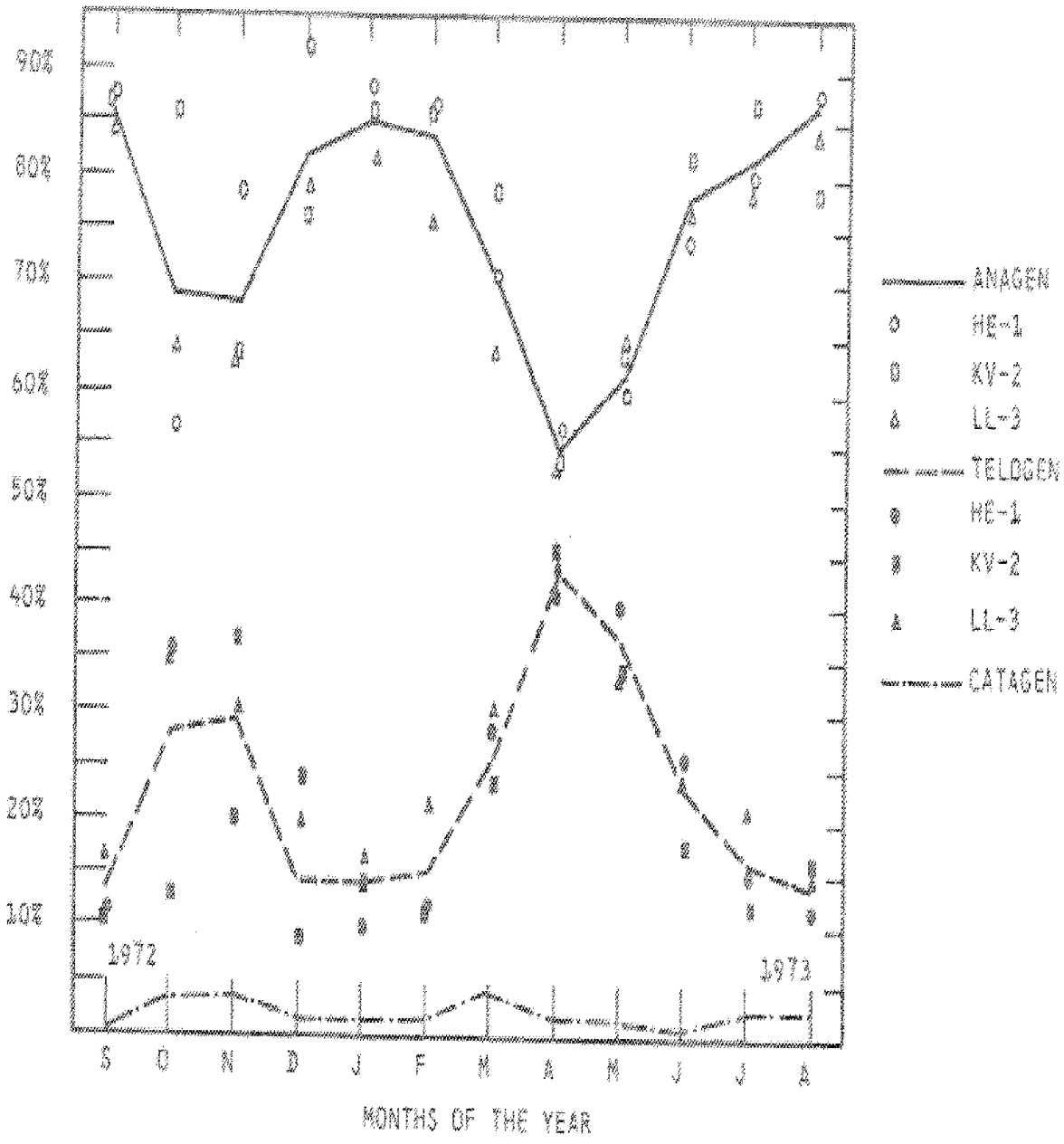
M - Months of age at time of biopsy.

FIGURE 15



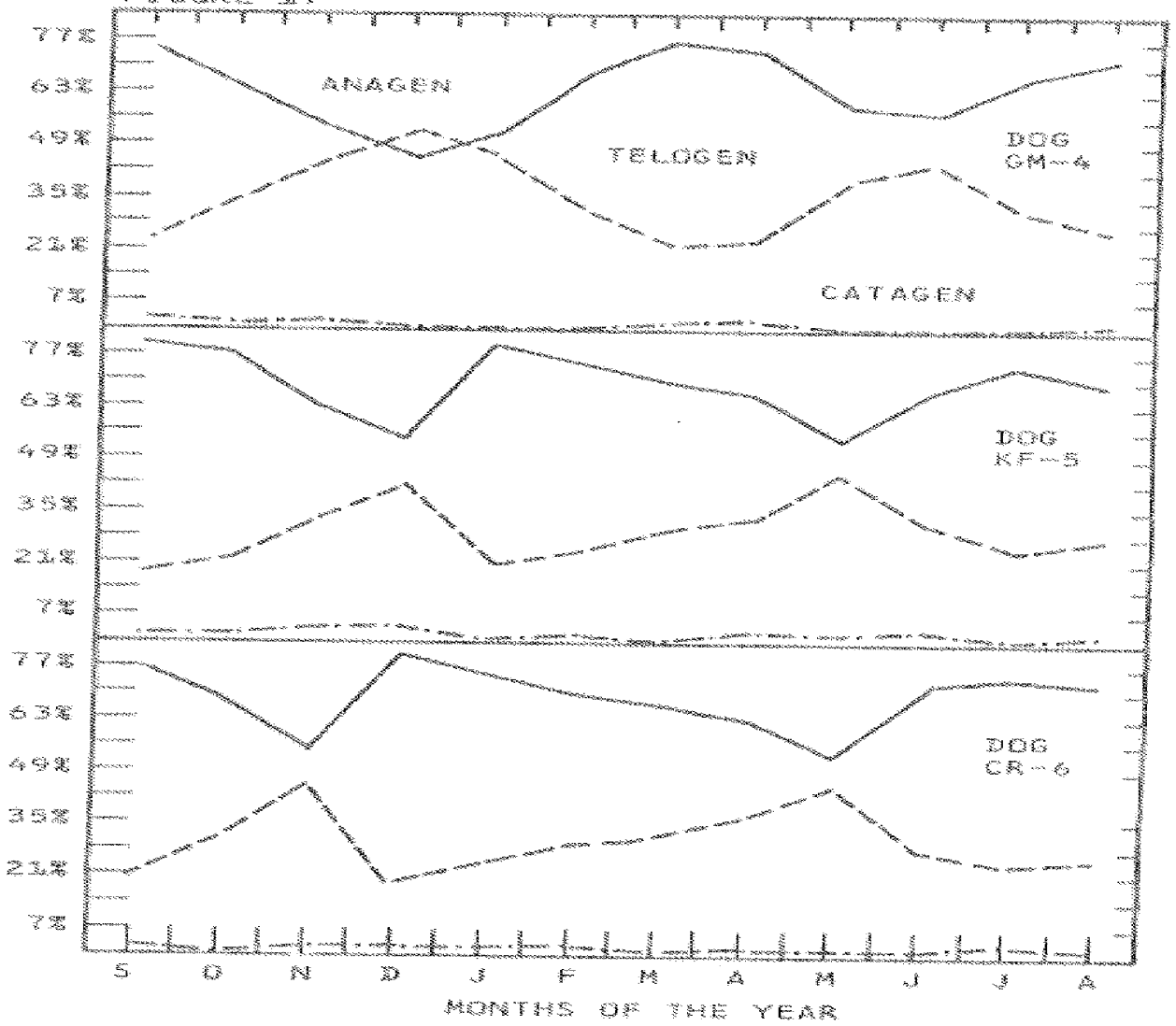
INDIVIDUAL PERCENTAGES OF HAIR CYCLE STAGES OF BOTH MAIN AND SECONDARY HAIR FOLLICLES DETERMINED DURING LIGHT MICROSCOPIC EXAMINATION OF SLIDES PREPARED FROM SKIN OF THE SADDLE REGION FROM THREE MALE BEAGLE DOGS, DURING THE FIRST YEAR OF LIFE.

FIGURE 16



MEAN PERCENTAGE OF HAIR FOLLICLE STAGES OF BOTH MAIN AND SECONDARY HAIR FOLLICLES FROM BIOPSIES OF THE SADDLE REGION OF THREE MALE BEAGLE DOGS, DURING THE FIRST YEAR OF LIFE.

FIGURE 17



PERCENTAGE OF ANAGEN, TELOGEN, AND CATAGEN OF BOTH MAIN AND SECONDARY HAIR FOLLICLES DETERMINED DURING LIGHT MICROSCOPIC EXAMINATION OF SLIDES PREPARED FROM SKIN OF THE SADDLE REGION FROM THREE MALE BEAGLE DOGS, DURING THE SECOND YEAR OF LIFE.

EXHIBIT D

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A study of normal hair growth in the dog

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ABSTRACT

The rate of hair growth in the forehead, shoulder and flank regions of four normal-coated, cross-bred, male dogs maintained under the same controlled conditions of photoperiod, ambient temperature and diet, was measured during two six-week periods, one during summer and the other in winter. It was found that the rate of hair growth varied between individual dogs and also between the different regions in the same dog. Growth rate was most rapid in the shoulder region, followed by the flank and then the forehead regions, and was slightly more rapid in the shoulder and flank regions of three of the dogs during summer than in winter. Possible explanations for these variations in hair growth rate are discussed.

INTRODUCTION

Although a certain amount of study has been devoted to hair growth in laboratory animals, man and domesticated animals of economic importance, especially sheep, little is known regarding the features of normal hair growth in the dog. Comben (1951) studied the pattern of hair growth in one body region of a single Greyhound dog over a period of two months in the early autumn in England. Al-Bagdadi (1975) reported on the hair growth of Beagle dogs during the North American summer and winter. Butler & Wright (1981) investigated the rate of hair growth in two male, two entire female and two spayed female Greyhounds in England over a two-year period, and correlated it with plasma progesterone and testosterone levels. Hale (1982) studied the periodic hair shedding (moulting) in a normal German Shepherd bitch over a period of five years also in England. Apart from these workers, no significant studies of hair growth in normal dogs have been reported.

The sight of a glossy, healthy-looking hair coat is one of the important aesthetic pleasures of dog ownership. Alopecia, unassociated with inflammatory skin

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disease, is not uncommon in the dog and may lead to considerable distress, not to say expense, to the owner, and may in extreme cases even lead to euthanasia of the animal. In order to understand the causes of hair loss in dogs and thus be able to prescribe rational therapy, it is essential that the various factors influencing hair growth are known. Before these factors can be investigated, the normal hair growth pattern of the dog must be ascertained.

This communication described our observations on hair growth in three body regions of four male dogs maintained under the same controlled conditions of photoperiod, ambient temperature and diet.

MATERIALS AND METHODS

Experimental animals

Four cross-bred, normal-coated (Gair, 1928), entire male dogs, aged between 18 months and 3 years, obtained locally, were used in this study. The animals were immunized against canine distemper, viral hepatitis and canine parvovirus infection, checked and found negative for the presence of microfilaria of *Dirofilaria immitis*, and were given suitable anthelmintic therapy one month before the study commenced. The dogs were caged individually in an animal house in which the ambient temperature was controlled between 18°C and 27°C, and where they were exposed to the same fixed length of photoperiod. The animals were fed a calculated amount of a commercial canned[1] and a dry[2] dog food at the same time each afternoon. Bodyweights were recorded weekly.

Experimental procedure

The same three areas of the body surface were selected for study in each dog, namely the forehead, the shoulder and the flank regions. These areas were clipped and wet-shaved, and a smaller area measuring 2·0 × 2·0 cm was marked out in each with a felt pen.

Each week, on the same day and at the same time, the marked areas were dry-shaved with a disposable safety razor, the shavings from each area being placed in a separate jar containing ethyl alcohol. Hair samples were then transferred to a Petri dish and with the aid of an illuminated magnifying glass[3], 10 primary hairs with cut ends signifying previous shaving, were selected at random and mounted individually in a straight position on a glass microscope slide with transparent plastic tape[4]. Each hair was then measured by reading the vernier scale on a travelling microscope. This procedure was repeated weekly for a period of six weeks and, after an interval of four months, for a further six-week period, one measurement period being during the summer the other in winter.

[1] 'Chum'.
[2] 'Meaty Bites' (Uncle Ben's, Australia).
[3] Maggylamp (Newbound & Co., Sydney).
[4] Sellotape.

All the dogs remained heal
course of the study. The ra
winter periods of measurer
shoulder and flank, of the fo
Fig. 1. It can be seen that t
also between the three selec
pattern of hair growth occ
growth occurred in the sh
forehead. This ranking of the
all the dogs both in summer

TABLE 1. Mean and standard dev

Dog	Weeks 1	Mean	S.D.
1	Summer	1·875	0·140
	Winter	2·195	0·075
2	Summer	2·302	0·186
	Winter	2·374	0·105
3	Summer	2·980	0·146
	Winter	2·759	0·151
4	Summer	2·542	0·140
	Winter	2·923	0·089
1	Summer	3·102	0·153
	Winter	3·342	0·118
2	Summer	3·865	0·094
	Winter	3·540	0·106
3	Summer	4·726	0·321
	Winter	4·076	0·096
4	Summer	5·428	0·374
	Winter	4·159	0·135
1	Summer	2·610	0·150
	Winter	2·567	0·121
2	Summer	3·339	0·148
	Winter	2·901	0·353
3	Summer	3·709	0·219
	Winter	1·853	0·214
4	Summer	4·108	0·098
	Winter	3·473	0·138

RESULTS

All the dogs remained healthy and their bodyweights constant throughout the course of the study. The rates of hair growth observed during the summer and winter periods of measurement in the three selected areas, viz. the forehead, shoulder and flank, of the four dogs are shown in Table 1 and diagrammatically in Fig. 1. It can be seen that the growth rates differed between individual dogs and also between the three selected regions in each dog, so that a distinct regional pattern of hair growth occurred. In decreasing rate order, the most rapid hair growth occurred in the shoulder region, followed by the flank and then the forehead. This ranking of the regions according to speed of hair growth is similar in all the dogs both in summer and winter. With the exception of Dog 1, hair growth

TABLE 1. Mean and standard deviation of rates of hair growth in the three body regions in the four dogs (mm)

		Weeks	1		2		3		4		5		6	
Dog			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Forehead														
1	Summer		1·875	0·140	2·057	0·109	2·275	0·193	2·297	0·354	2·192	0·050	2·342	0·108
	Winter		2·195	0·075	2·452	0·074	2·444	0·082	2·643	0·057	2·537	0·126	2·482	0·116
2	Summer		2·302	0·186	2·516	0·136	2·320	0·073	2·319	0·105	2·295	0·088	2·483	0·157
	Winter		2·374	0·105	2·474	0·085	2·460	0·082	2·489	0·095	2·410	0·095	2·548	0·071
3	Summer		2·980	0·146	3·132	0·054	2·841	0·064	2·789	0·081	2·672	0·109	2·557	0·135
	Winter		2·759	0·151	2·878	0·103	2·662	0·091	2·622	0·071	2·987	0·053	2·793	0·105
4	Summer		2·542	0·140	2·802	0·071	2·956	0·272	2·885	0·114	2·764	0·141	2·672	0·130
	Winter		2·923	0·089	2·967	0·071	2·854	0·105	2·780	0·111	2·901	0·102	2·806	0·0962
Shoulder														
1	Summer		3·102	0·153	3·338	0·114	3·833	0·210	2·900	0·291	2·892	0·177	2·377	0·247
	Winter		3·342	0·118	3·337	0·125	3·420	0·093	3·375	0·132	3·793	0·123	3·945	0·082
2	Summer		3·865	0·094	4·576	0·142	4·339	0·319	3·927	0·181	3·877	0·158	3·807	0·154
	Winter		3·540	0·106	3·555	0·101	3·563	0·098	3·395	0·081	3·750	0·098	3·945	0·082
3	Summer		4·726	0·321	5·069	0·209	5·037	0·368	5·010	0·132	4·773	0·179	4·587	0·154
	Winter		4·076	0·096	4·580	0·081	4·548	0·081	4·570	0·104	4·543	0·146	4·001	0·118
4	Summer		5·428	0·374	6·739	0·210	5·515	0·495	5·185	0·225	4·941	0·181	4·863	0·229
	Winter		4·159	0·135	4·870	0·103	5·081	0·104	4·620	0·450	4·638	0·096	4·073	0·097
Flank														
1	Summer		2·610	0·150	2·755	0·160	2·860	0·131	2·677	0·104	3·065	0·075	2·616	0·138
	Winter		2·567	0·121	2·465	0·114	2·768	0·105	2·944	0·111	3·480	0·087	3·308	0·093
2	Summer		3·339	0·148	3·692	0·147	3·496	0·220	3·231	0·148	2·654	0·291	3·308	0·132
	Winter		2·901	0·353	3·121	0·066	3·064	0·082	2·981	0·127	3·237	0·062	3·181	0·049
3	Summer		3·709	0·219	4·067	0·174	3·860	0·131	3·596	0·132	3·575	0·128	3·436	0·113
	Winter		1·853	0·214	2·069	0·130	2·043	0·067	2·081	0·086	2·098	0·064	2·086	0·061
4	Summer		4·108	0·098	4·477	0·163	4·071	0·173	3·883	0·126	3·838	0·145	3·745	0·243
	Winter		3·473	0·138	3·748	0·128	3·743	0·122	3·179	0·150	3·227	0·079	3·053	0·121

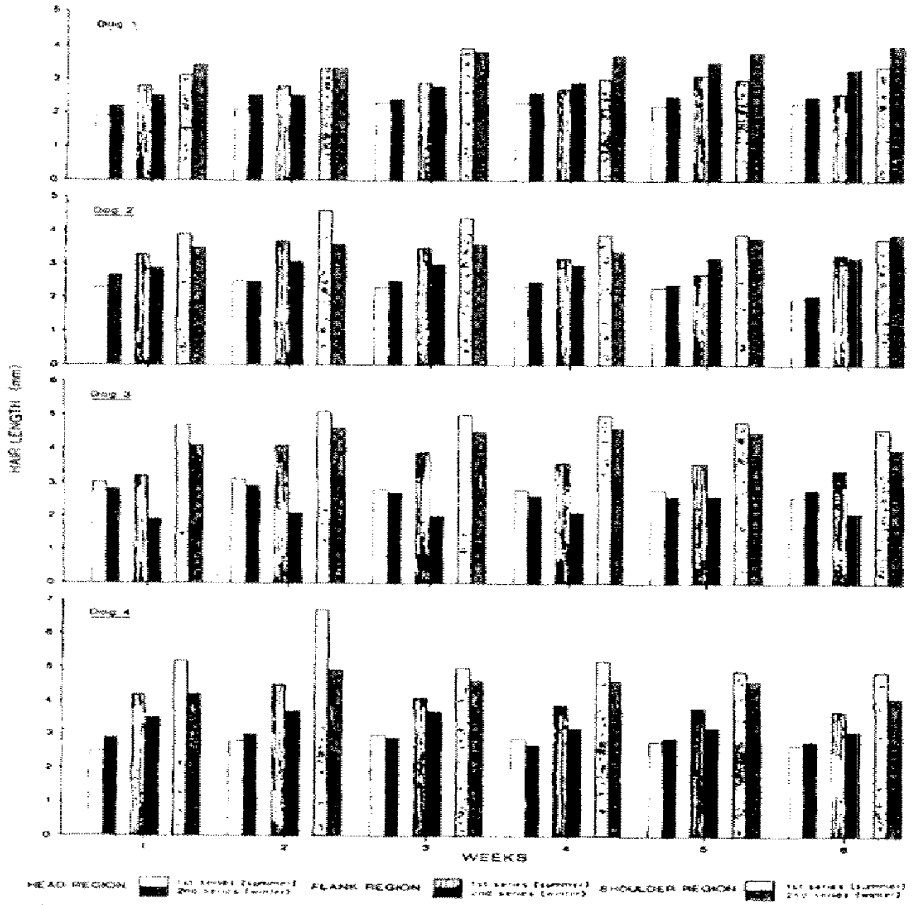


FIG. 1. Histogram showing the growth rates in the three regions in the four dogs in summer and winter.

rates were slightly more rapid in the shoulder and flank regions in summer than in winter.

In each region in all dogs during summer and winter, in the first week after initial shaving, the hair grew to only about 80–90 per cent of the length achieved in the second and subsequent weeks. Peak growth occurred during the second week and

TABLE 2. Average range (mm) in

Region	
Forehead	Summer Winter
Shoulder	Summer Winter
Flank	Summer Winter

this was followed by a slight remainder of the observation region of all the dogs, the regi

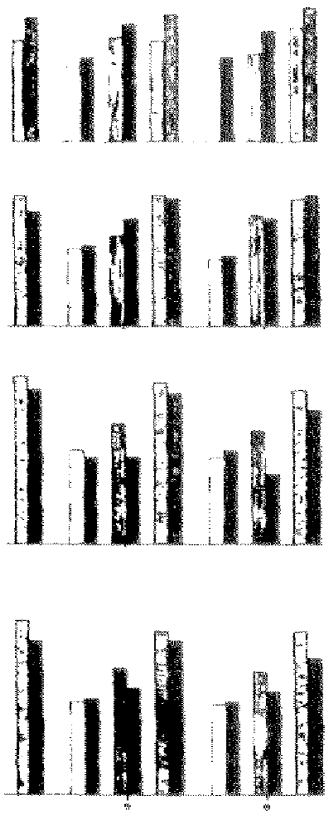
Table 2 shows the weekly hair growth rates observed in that in the forehead region t 0·31 to 0·37 mm, in the flan 0·49 to 0·71 mm.

Statistical analysis of the r

(a) the rate of hair growth that of the other three
(b) there was a significan summer and winter, th
(c) the rate of hair growt of the dogs during sum

This study has shown that th the dog and these variations factors affecting hair growth

In most mammals hair ten active growth (anagen) follc variable period of time by papilla is pinched off and the from which it is easily epilat hair, but not the axillary o appear to be a resting pe



? SHOULDER REGION

three regions in the four dogs in

flank regions in summer than in

inter, in the first week after initial
ent of the length achieved in the
rred during the second week and

TABLE 2. Average range of hair growth rate and mean average hair growth rate (mm) in the three body regions of the four dogs

Region		Average range of hair growth (mm)		Mean average rate of hair growth (mm)	
		Weekly	Daily	Weekly	Daily
Forehead	Summer	1·2–3·1	0·17–0·44	2·2	0·31
	Winter	2·1–3·0	0·30–0·42	2·6	0·37
Shoulder	Summer	3·0–6·7	0·42–0·96	5·0	0·71
	Winter	3·3–4·9	0·47–0·70	4·1	0·49
Flank	Summer	2·3–4·5	0·33–0·64	3·4	0·49
	Winter	1·9–3·7	0·27–0·53	2·8	0·4

this was followed by a slight falling off in the rate which then became steady for the remainder of the observation period. This effect was most marked in the shoulder region of all the dogs, the region where the most rapid growth rate took place.

Table 2 shows the weekly and daily average range and the mean average rate of hair growth rates observed in each of the three sites in all four dogs. It can be seen that in the forehead region the range of the mean average daily hair growth was 0·31 to 0·37 mm, in the flank region 0·40 to 0·49 mm and in the shoulder region 0·49 to 0·71 mm.

Statistical analysis of the results enables the following inferences to be drawn:

(a) the rate of hair growth of each individual dog was significantly different from that of the other three dogs ($p = 0{\cdot}025$),

(b) there was a significant difference between the means of the growth rates in summer and winter, the former being more rapid ($p = 0{\cdot}025$).

(c) the rate of hair growth varied significantly in the three body regions in each of the dogs during summer and winter.

DISCUSSION

This study has shown that there are marked variations in the rate of hair growth in the dog and these variations must be taken into account in any future studies of the factors affecting hair growth in this species.

In most mammals hair tends to grow in a cyclical fashion, i.e. there is a period of active growth (anagen) followed by a rest period. The latter is followed after a variable period of time by telogen, in which the hair follicle shortens, the hair papilla is pinched off and the hair becomes a club hair, lying passively in the follicle from which it is easily epilated. In sheep, the wool, and in man the scalp and beard hair, but not the axillary or pubic hair, grow continuously and there does not appear to be a resting period, although telogen occurs and hairs are shed

periodically. In dogs and most other domesticated animals, the hair grows until it attains its pre-ordained length, which varies according to body region and is genetically determined, and then enters a resting phase, which may last for a considerable time before telogen occurs and the hair is shed. Hair replacement in the dog is similar to that seen in the cat and in man, in which a mosaic pattern occurs with neighbouring follicles being in different stages of the hair cycle at any one time (Muller & Kirk, 1976). This means that only certain of the follicles in a given area are in telogen and so hair loss and replacement occurs in a scattered manner and the area does not lose all its hair covering at once. At times of moult, usually spring and autumn, hormonal changes stimulated by variations in photoperiod accelerate the hair cycle, so that more hairs arrive at telogen and are shed than during the remainder of the year.

Durward & Rudall (1949) suggested that hair replacement varies in different animals and each species has its own particular pattern which remained constant. This pattern is determined by the type of follicle activity, which according to these authors might be one of three, viz. (a) continuously active as in wool and human scalp and beard hair, (b) periods of activity and inactivity with neighbouring follicles in different phases of the hair cycle, and (c) periodic activity with neighbouring follicles in the same phase. Hair growth in the dog would seem to fit into type (b), although Comben (1951) considered that the Greyhound was suitable for inclusion in type (c). The latter type of hair replacement is characteristic of rodents in which a wave pattern occurs.

Each region of the dog's body has its own ultimate length of hair beyond which no further growth occurs. This phenomenon is responsible for the distinctive coat lengths of the various breeds of dog and must be genetically determined. A short hair coat is dominant to long, and straight or wavy types are recessive or partially so to wire coat types (Al-Bagdadi & Lovell, 1974). The mechanism whereby these different regional and breed hair lengths are attained may be either variation in the duration of anagen, or differences in the rate of hair growth, or a combination of the two. This study has shown that there are very different rates of hair growth in the three sites observed and the speed of growth appears to be related to the ultimate length of the hair in each particular site. For example, in the shoulder region of the experimental dogs where the ultimate hair length was about 30 mm, the average rate of hair growth was 6·7 mm/week, whereas in the forehead region with an ultimate hair length of about 16 mm, the growth rate was 2·8 mm/week. Even so, it is difficult to believe that the great differences in hair length that occur in the various body regions of some canine breeds, e.g. compare the hair length on the face of an Afghan Hound with that on its body and limbs, could be due solely to differences in speed of hair growth between regions. The possibility that differences in the anagen period of the hair cycle occur in the various regions in addition to the demonstrated differences in hair growth rate, is supported by the fact that if an Afghan Hound is clipped, it takes about eighteen months to two years for the body and limb hair to attain its pre-ordained length, while the face hair achieves it within a matter of weeks.

Regional differences in ha
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Although the experimental
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It is difficult to explain the
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of five weeks (Seymour, 1926)
after shaving the rate of hair
occurring later but this effect
became longer. He postulated
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However, repeated observation
compared to true shaving of th
et al. (1967) studied the effe
seven-day intervals, and found
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In the present study the wee
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r is shed. Hair replacement in
in, in which a mosaic pattern
stages of the hair cycle at any
nly certain of the follicles in a
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ng at once. At times of moult,
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ivity, which according to these
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enetically determined. A short
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i may be either variation in the
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For example, in the shoulder
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Regional differences in hair growth rate have also been reported in rats (Johnson, 1958) and man (Rothman, 1954; Saitoh *et al.*, 1967). Rothman reported that the eyebrow hairs have a shorter growing and a longer resting period than hairs from other parts of the body.

Although the experimental dogs in the present study were all classified as 'normal coated' (Gair, 1928), they showed individual differences in their hair growth rates, and, in fact, Dog 1 showed an inverse pattern of summer and winter growth rates when compared with the other three animals. Muller & Kirk (1976) have also noted that 'normal coated' breeds of dogs have different rates of hair growth which vary from breed to breed, and also between individual animals within a breed. These differences are again probably genetically determined and it was noted during the present study that Dog 1 was a shy, highly nervous and excitable animal compared with its fellows. This dog may have had a higher metabolic rate or higher cortisol output, which could have affected hair growth.

It is difficult to explain the slower growth rate, seen in all the regions in all the dogs in both summer and winter, which occurred in the first week after initial shaving. Comben (1951) observed a slow growth rate for the initial 32 days of his study, while he attributed either to the early observation period corresponding with the end of a resting period, or to a reaction to shaving. He tended to discount the latter in view of the reported findings on the effects of shaving on hair growth rate. It would seem unlikely that in the present study, in which the slow growth occurred only during the first week in all four dogs and during summer and winter observation periods, the first week coincided with the end of a resting period in all three body regions. Studies on the effects of shaving on hair growth are equivocal and often contradictory. Berthold (1850) found that hair growth was greater if the hair was shaved frequently. Although Trotter (1923) could find 'no effect on the growth of hair from shaving', her tabled results show that in general hair grew more rapidly during the first week after shaving than it did in the second week and more rapidly the first five weeks after cutting than it did during the second similar period of five weeks (Seymour, 1926). Seymour (1926) found that in the first few hours after shaving the rate of hair growth was much more rapid than the growth occurring later but this effect decreased progressively as the time since shaving became longer. He postulated that the more rapid growth immediately following shaving was a result of the hyperaemia induced by the irritation caused by shaving. However, repeated observations, comprising 'false shaving' of one side of the face compared to true shaving of the other side, failed to support this hypothesis. Saitoh *et al.* (1967) studied the effect of shaving the chest hair of 11 men twice, at seven-day intervals, and found no difference in the growth rate before and after shaving.

In the present study the weekly hair growth rate was decreased after the initial shaving but then achieved a peak rate in the week following the second shaving. Following the peak the growth rate declined slightly and remained at this level for the remaining portion of the observation period. It is difficult to conceive of any factor associated with shaving which inhibits growth in the first week, stimulates it

in the second week and then slightly inhibits again in the third week and finally has no apparent effect for the remaining three weeks. However, one possibility is that the first shave causes sufficient inflammation to depress growth, the second, when the skin has become a little more hardened by exposure to the environment, produces a hyperaemia stimulating growth; the third shave provokes less hyperaemia due to further skin hardening and so less stimulation, and the remaining shaves have little effect on the now much tougher skin. When observations were recommenced after a four month rest period the same sequence of events occurred.

The rate of hair growth in the shoulder and flank regions was greater in summer than in winter in three of the four dogs in the present study. This observation conflicts with those of Comben (1951) who reported that the daily rate of hair growth in the shoulder region of his Greyhound was 0·04 mm in summer and 0·18 mm in winter, and of Al-Bagdadi (1975) who found that the average rate of daily hair growth in male Beagle dogs was 0·40 mm in winter and 0·34 mm in summer. However, our observations agree with the findings of Butler & Wright (1981) that entire male and female Greyhounds showed maximal hair growth in mid-summer with virtually no growth occurring in late winter and late summer. These authors considered that low environmental temperatures depressed hair growth in late winter. Similar findings have been reported in cats (Baker, 1974; Ryder, 1976), cattle (Dowling & Nay, 1960) and man (Berthold, 1850). Such seasonal variations in hair growth rates may be related to either ambient temperature or duration of photoperiod, or to a combination of both. It has been suggested that there is a relationship between external temperature and the activity of the endocrine glands which affect hair growth (Seymour, 1926). Butler & Wright (1981) noted that the environmental effect was most marked in entire animals, supporting Seymour's suggestion, and, while there was still a seasonable variation in neutered bitches, the effect was much less marked and of shorter duration. They conducted observations during two successive years and found that while the animals showed no hair growth in the period mid-January to mid-February 1979, during the same period the following year, the two spayed and one of the entire bitches showed a growth spurt followed by a fall in rate to one similar to the previous year. The only obvious change between the two periods was that of environmental temperature, the mean of which was higher in 1980 than in 1979. Johnson (1977) suggests that the seasonal rhythm of hair growth is linked with changes in photoperiod in many species, the effect being mediated through the eyes on the hypothalamus. In the present study, the length of the photoperiod was kept constant the only variable between summer and winter being the higher ambient temperature in the former. As the environmental temperature was controlled within a certain range in the animal house, it may be that more marked differences in seasonal hair growth would have been produced if the dogs had been housed outdoors. It seems probable that normally seasonal variations in hair growth rate are the result of both photoperiod and ambient temperature changes among other factors. Butler &

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Wright (1981) suggest that 'there are seasonal effects on hair growth that are unrelated to those caused by seasonal variations in hormone levels and ... environmental temperature may be one such factor'.

It is apparent that further studies on hair growth in normal dogs are required, particularly in relation to the effects of photoperiod and ambient temperatures on the rate of hair growth. Such studies, however, requiring observations over long periods of time and involving large numbers of dogs, are very demanding in terms of financial and time commitments.

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EXHIBIT E

Comben et al., Observations On The Mode of Growth of the Hair of the Dog,
Br Vet J 1951 May 107 (5) 231-235

OBSERVATIONS ON THE MODE OF GROWTH OF THE HAIR OF THE DOG

By NORMAN COMBEN, B.Sc., M.R.C.V.S.,
Kensington.

Introduction

THE literature pertaining to the mode of growth of the hair of the domestic animals is very scant, and that work which has been recorded may be summarised as follows: (1) The hair pattern of various species has been studied by Kidd (1903) and by Wood-Jones (1924); (2) the periodic nature of hair growth in the rat by Butcher (1934), by Haddow, *et al.* (1945), and by Durward and Rudall (1949); (3) seasonal variations in the growth of the hair of the horse, by Hayes (1903), of the Arctic fox by Pocock (1912), and of the horse, cow and donkey by Duerdon and Whitnal (1930); (4) the effect of various physical conditions on the mode of growth of the hair of the guinea-pig by Strangeways (1933 a, b), of the Merino sheep by Bosman (1935), and of the human beard by Eatoh and Eaton (1937) and Kayser (1938); (5) the mode of growth of the hair on skin grafts in the rat by Butcher (1936, 1937); (6) the effect on the hair growth of a bitch suffering from hyperplastic endometritis by de Vita (1939) and of dogs receiving massive doses of œstrogens by Gardner and de Vita (1940); and (7) the classification of coat types according to the texture of the hairs by Robb (1946) and Joshua (1946), and according to the colour of the hairs by da Fonseca and Cabral (1945).

It is thus apparent that no significant studies have as yet been reported on either the mode or the rate of growth of the hair of the dog. The need for such a study became apparent when, in 1945, the "Dyed Greyhound" case was reported by Kirk. At the trial of this case it was stated that the hair of the greyhound grows at the rate of approximately one-half of an inch per month; a study of the literature regarding other species, however, suggested that the matter might not be as simple as this.

Wilson (1949) has stated that more knowledge on this subject would materially assist in the enforcement of the Kennel Club regulations concerning the dyeing of dogs' coats at shows.

Material and Methods

Two series of measurements were consequently undertaken on an entire, dark brindle, adult male greyhound housed at the Royal Veterinary College. The series which is presented below ran from 26.9.49 to 28.11.49.

The hair was clipped and carefully wet-shaved over an area 1½ inches long and 2 inches wide, the length of the area being parallel to, and about 2 inches below, the spine. The reason for the choice of this site will be explained later.

Each seventh day about one inch of this area was reshaved (dry) with a hollow-ground razor, the shavings were collected and washed in absolute alcohol,

and, after being well shaken, a random sample was taken and mounted under a cover slip on a large slide.

The method of measuring consisted of the direct measurement of 100 hairs from each sample, using a microscope with a 3-inch objective and a × 5 eye-piece carrying a 10 mm. × 100 scale, which had been previously calibrated against a millimetre scale placed on the stage.

Results

The growth rates observed in the two series of measurements were essentially similar, but the standard deviation was smaller in the second series. Since it is considered that this was due simply to a general improvement in technique, only this series is presented.

Means of 100 measurements of hair length at 7-day intervals. 2nd series.

Date.	*Mean of 100 measurements.* (mm.)
26. 9.49	—
3.10.49	0.327
10.10.49	0.603
17.10.49	0.882
24.10.49	1.035
31.10.49	2.85
7.11.49	3.18
14.11.49	4.84
21.11.49	5.92
28.11.49	8.03

The mean lengths are plotted against a time scale in the figure. It will be seen that two distinct rates of growth of hair are demonstrated, an initial slow growth of about 0.04 mm. per day and a second more rapid growth period of about 0.18 mm. per day.

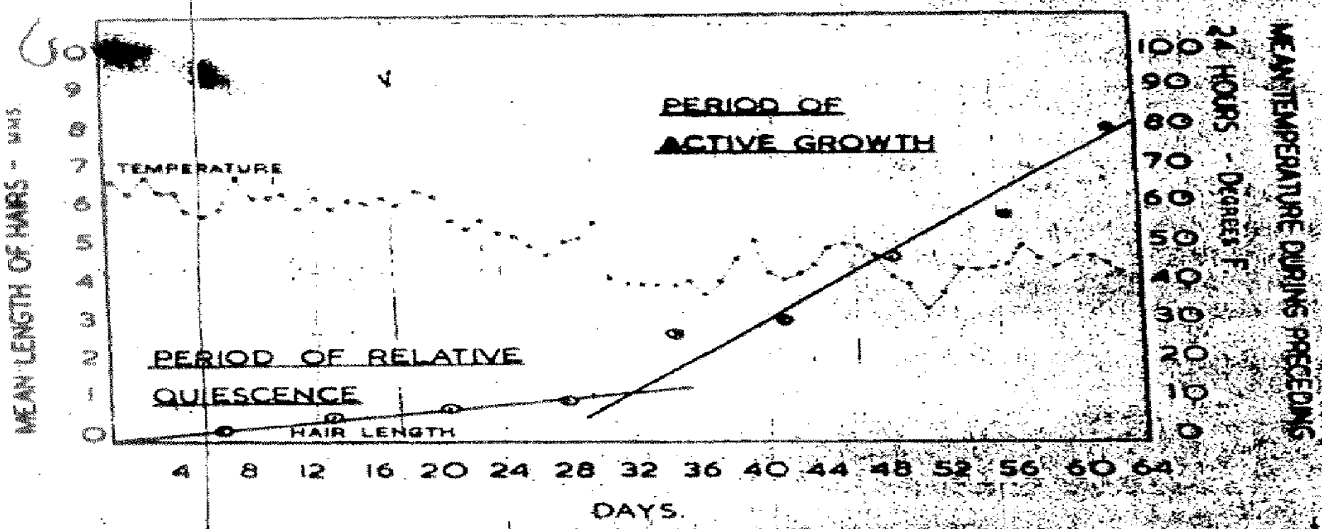
Discussion

In 1934, Butcher reported the phenomenon of cyclical hair growth in the rat, having found that at any one time certain hair follicles in the skin are active and others are in a resting phase, and that a wave of growth passes slowly over the body. This has since been most beautifully confirmed by Haddow, *et al.* (1945), who noticed a marked symmetry of certain hair reactions in the rat and photographed the advance of this growth wave after absorption of an alloxazine dye by the active growing follicles following intraperitoneal injection. They found, broadly speaking, that this growth wave passed from the mid-ventral line upwards on either side of the body towards the mid-dorsal line. Thus areas of skin on a line parallel with the long axis of the body would be expected to be in the same phase of growth at any one time. It was in anticipation of an analogous situation in the animal under investigation that the area of skin previously described was chosen. Haddow, *et al.*, reported, furthermore,

that these cycles of growth could be affected by factors such as nutrition, lactation, season and age.

Durward and Rudall, in a further study of factors which may affect the growth pattern (1949), state: "The mode of hair replacement varies in different animals and has a constancy in relation to species. Three different types of follicle activity determine the various patterns: (1) Continuously active, as in wool and human hair; (2) periods of activity and inactivity with neighbouring follicles in different phases; and (3) periodic activity with neighbouring follicles in the same phase."

GROWTH OF HAIRS OVER 63 DAY PERIOD WITH DAILY ATMOSPHERIC TEMPERATURES.



The results presented in the figure demonstrate two distinct rates of growth of hair in the dog, which we may reasonably assume to represent an initial phase of relative quiescence, followed by a phase of activity, of the hair follicles. It is impossible to be sure without further study whether the explanation for this is (1) that the period studied covered the end of a resting period and the beginning of an active period, or (2) that the growth picture recorded is the initial reaction to shaving. In view of the findings in other species this explanation is unlikely. Also, in numerous preliminary trials on small areas of the [illegible] was obtained that shaving is followed by some degree of stimulation of hair growth and not inhibition. This is in confirmation of the findings of Bonnet (1926) and Strangeways (1933), and in contradistinction to the findings of Trotter (1928).

It would seem, therefore, that hair growth in the greyhound is probably in some manner a periodic and possibly a cyclical phenomenon and suitable for inclusion in Group 3 of Durward and Rudall. Certainly the results obtained serve to stress the inadvisability of stating any dogmatic figure as representing the rate of growth of hair in this species with our present knowledge. We must first learn far more concerning the hair growth pattern, the factors which can influence this pattern, and the mechanism of moulting in the various breeds.

Such investigations are extremely tedious, however, and require the use of a large number of animals which may be observed continuously for a period of not less than a year.

Summary

1. A review is made of the literature concerning the mode of growth of the hair of the domestic animals, and the importance of a better understanding of this phenomenon in the dog is stressed.

2. Measurements of the length of hairs of a greyhound at 7-day intervals following shaving are described.

3. The suggestion is made that hair growth in the dog follows a periodicity pattern of alternating phases of rest and activity.

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My thanks are due in the first place to the Animal Health Trust for the award of a Research Training Scholarship, during the tenure of which these observations were made; to Professor J. B. Buxton, principal of the Royal Veterinary College, for facilities placed at my disposal at this institution; to Professor E. C. Amoroso for drawing my attention to this problem, and for numerous helpful suggestions; and to Miss M. B. Dodds, S.R.N., for drawing the figure.

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THE CONTRIBUTION OF THE PHARMACEUTICAL INDUSTRY TO VETERINARY MEDICINE

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It has been well said: "Wisdom is knowing what to do next; Skill is knowing how to do it, and Virtue is doing it." There may be many who possess all three of these desirable attributes in some measure, and if the quotation be used to illustrate three phases of the development of the modern veterinary product, it is not to suggest that those who play their part have either a monopoly of the one or a deficiency of the other two. Let us pay tribute to the wisdom of those men and women research workers whose labours enable us to know what to do next to solve the problems of our day; to the skill of the pharmacist, and others within this great industry, who know how to manufacture and distribute the products of research; and to the veterinary surgeon, who alone, by virtue of his training and experience, can give effect to the work of the others.

The record of service of the pharmaceutical houses to the profession is an honourable one and in a number of instances many generations of veterinary surgeons have been served with unbroken continuity by the same firm—Willows Francis was founded in 1751, Wyleys some time prior to 1757, and Hewlett's in 1832, to name but three.

The Traveller

Not all of us are old enough to remember, from our personal experience, the traveller who called on behalf of such firms in bygone days. This was before the word "representative" had been thought of, and, indeed, it would not have suited him at all, for he was an ambassador, no less, and his object was first to establish friendly relations with his client and then become the well-loved friend of the entire family. Business was not neglected, for his loyalty to his firm was implicit, but it took place at the proper time, which was the end of his visit. The contents of the shelves would be rapidly surveyed by his experienced eye.